SUBCAGE COLLECTION AND TREATMENT OF AQUACULTURE WASTES FROM THE FRESHWATER CAGE PRODUCTION OF RAINBOW TROUT

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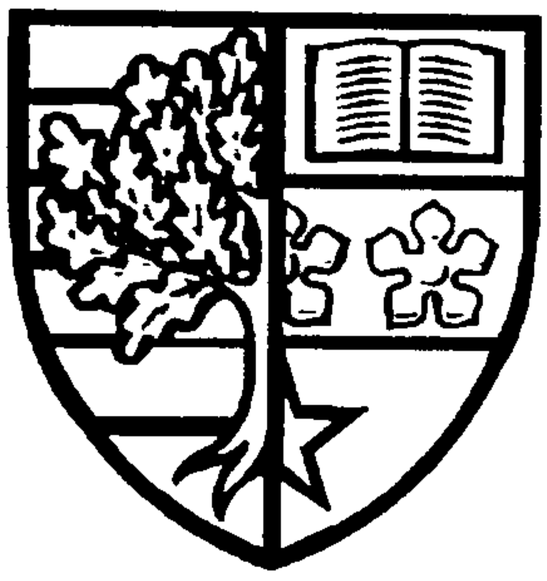

Some information derived from this thesis is also used in the following paper:

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Nomenclature

BOD	Biological oxygen demand	mg l^{-1}
COD	Chemical oxygen demand	mg l^{-1}
COD_{rem}	Chemical oxygen demand removed	mg l^{-1}
TP	Total phosphorus	
DRP	Dissolved reactive phosphorus or orthophosphate	
TAN	Total ammonia nitrogen	
FCR	Feed conversion ratio	
DO	Dissolved oxygen	
SS	Suspended solids	mg l^{-1}
TS	Total solids	mg l^{-1}
DAF	Dissolved air flotation	
VS	Volatile solids	mg l^{-1}
VSS	Volatile suspended solids	mg l^{-1}
SRT	Solids retention time, $1/\theta$	days
HRT	Hydraulic retention time, θ	days
UASB	Upflow anaerobic sludge blanket reactor	
CSTR	Continuously stirred tank reactor	
SBR	Sequencing batch reactor	
r_g	Rate of bacterial growth (parameter in *eq 2.4*)	g l^{-1} day^{-1}
μ	Specific growth rate	$time^{-1}$
X	Biomass concentration	g VSS l^{-1}
S	Concentration of limiting substrate	
	(parameter in *eq 2.6*)	g l^{-1}
μ_m	Maximum growth rate	$time^{-1}$
K_s	Half saturation constant	

Y	Yield coefficient or fraction of substrate converted to biomass	gVSS g substrate^{-1}
Q	Volumetric flowrate	m^3 day^{-1}
X	Mass concentration of microorganisms (parameter in *eq 2.10*)	g kg^{-1}
S_i, S	Incoming and outing substrate concentrations (parameters in *eq 2.12*)	kg m^{-3}
V	Volume	m^3
k_d	Rate of endogenous decay	$days^{-1}$
BMP	Biochemical methane potential	
CH_{4max}	Ultimate methane yield	l
CH_4	Cumulative methane yield at time t (parameter in *eq 2.19 & 2.20*)	l
k	First order rate constant	
VFA	Volatile fatty acid	
FC	Faecal coliforms	
FS	Faecal Streptococci	
SMP	Standard Method Procedure	
MIW	Mean Individual Weight	
NY	Nylon net	
WS	Wind shear net	
SN	Synthetic net	
ALK	Alkalinity	mg $CaCO_3$ l^{-1}
TDS	Total dissolved solids	mg l^{-1}
ADCP	Acoustic doppler current profiler	
DGPS	Differential geographical positioning system	
ERM	Enteric redmouth disease	

SD	Standard deviation	
T1	Thermophilic anaerobic digester containing 25% cattle slurry, 75% aquaculture effluent feed composition	
T2	Thermophilic anaerobic digester fed 10% cattle, 90% aquaculture effluent feed composition	
T3	Thermophilic anaerobic digester fed aquaculture effluents only	
UAN	Unionised ammionia	
pKa	dissociation constant for species a	
FI	Feed input	kg
Fd	Food wastage, dry weight basis	kg
Fd_{dig}	Feed digestibility	%
Fd_{was}	Feed wastage or percentage of total feed input not consumed by fish in cage system	%
Fd_{wat}	Feed moisture content	%
Fae	Faecal matter production	kg
CHP	Combined heat and power	
NPV	Net present value	
d	Discount rate (parameter in *eq. 8.3*)	%
n	Number of years discounting	
IRR	Internal rate of return	

Abstract

The growth of the intensive aquaculture industry, due mainly to a decline in wild fisheries stocks, has focussed attention on the environmental impacts of aquaculture activities. More recently, interest in the impact of freshwater cage production on lake sediments and water quality has emerged, and in Scotland, now forms part of the regulatory assessment on larger loch based trout farms. The main objectives of this thesis were to reduce the impact of solid material from the freshwater cage production of rainbow trout by means of undercage collection and its subsequent biological stabilisation through anaerobic digestion.

A subcage collection device was designed, installed and monitored at a Scottish freshwater cage aquaculture site, situated in Loch Earn, a relatively large lake in central Scotland. Measurement of surface (0 – 4 m) water current velocities at the study site revealed they had the potential to exceed the sedimentation velocity of solid waste particles resulting in the lateral dispersion of waste from the cage. As a result, the solid waste collection efficiency was less than 1%.

The pollution potential of food derived solid waste effluent from the aquaculture process was significantly reduced by means of anaerobic digestion. The chemical oxygen demand of the waste was reduced by up to 80% and this could be further enhanced to 90% with the employment of pre and post-treatment methods. Anaerobic digestion also had the potential to inactivate a typical fish bacterial disease. An economic analysis, however, revealed that the quantity of waste material produced from a typical Scottish farm was not sufficient to generate enough biogas to make the system cost effective. Co-digestion with cattle slurry was found to be feasible with no inhibitory effects on the digestion process.

Introduction to Thesis

The overall aims of the study were to determine whether or not the collection and disposal of solid wastes lost from freshwater cage fish farms in Scotland is technically and economically feasible. The reduction in waste lost to the environment from cage fish farms is an essential part of the sustainable development of freshwater cage fish farming. Similarly, disposal of waste products from aquaculture should ideally ensure the least adverse and most beneficial impact on the environment.

The growing importance of the aquaculture industry in relation to world fisheries and its impact on the environment is discussed (*Chapter 1*). The role of waste management in the aquaculture industry is also examined together with a literature review of the technologies used in this study (*Chapter 2*).

The performance of the designed undercage waste collection system for cage aquaculture was monitored at a pilot scale on a commercial freshwater fish farm in Scotland (*Chapter 4*). Laboratory scale anaerobic digestion systems were used to evaluate the potential for stabilising aquaculture wastes (*Chapter 5*). The optimal conditions for the anaerobic treatment of aquaculture wastes by anaerobic digestion were investigated (*Chapters 6, 7*).

Furthermore, the economic viability of subcage collection and anaerobic digestion as a means of waste treatment were examined (*Chapter 8*).

Chapter 1

Introduction to Global Aquaculture

It is estimated that water comprises more than 70% of the total surface area of the earth (Horne and Goldman, 1994; Bardach, 1997). All water, whether marine, brackish, or fresh, is populated with living matter. The most common of which are bacteria, fish, crustaceans, molluscs and plant life (Barnabè, 1994). The harvest of aquatic creatures for human consumption has long been established. Although the evolution of agriculture and animal husbandry has meant the relative importance of aquatic protein sources has declined, fishery products still represent an important global source of animal protein, estimated at approximately 16% of the total protein intake for the human diet. This figure is strongly influenced by geographical, economical and cultural differences throughout the world (Bardach, 1997). Developed countries or more prosperous societies are less dependent on protein derived from aquatic animals in contrast with developing countries where meat is less plentiful. Aquatic sources, for example, provide Asia with approximately 29% of dietary protein in comparison to 9% in Latin America where beef is the traditional source of essential amino acid intake (FAO, 1999).

1.1. THE GLOBAL FISHERIES MARKET

The capture fisheries industry has long been the predominant means of satisfying the world demand for fish. The fishing industry, as with other agriculturally related industries, has moved increasingly towards the intensive production of species. The practice of rearing, growing or producing organisms in water under controlled or semi controlled conditions is known as aquaculture (Meade, 1989; Barnabè, 1994). It can also be best described as the aquatic counterpart of agriculture, the farming of aquatic

organisms is similar to that of animal husbandry practices, with the aim of enhanced production through some form of intervention or manipulation of the organism life cycle (FAO, 1995; Beveridge, 1996). The cultivation or aquaculture of aquatic creatures dates back to China at least 4,000 years ago. However it has not been until the early part of the 20th century that the aquaculture industry started to develop into the intensive business it is today as a result of scientific and technological breakthroughs in breeding and husbandry practices (Shepherd and Bromage, 1992; Beveridge, 1996).

The global production of fish through capture fisheries has grown from 18 million tonnes (t) in 1950 at a rate of 6% until 1969 at which point the average rate of increase declined to 2% per year falling to almost zero in the 1990's (Figure 1.1). By the end of the 1990's, however, an estimated 50% of major marine fish stocks were fully exploited, about 15% already overfished and another 7% depleted (FAO, 2000). Declining capture fisheries have been attributed to a number of factors primarily the overfishing of stocks together with the development of Economic Exclusion Zones (EEZs) and the increase in fuel prices (Shepherd and Bromage, 1992; Beveridge, 1996). The decline in growth rate of capture fisheries is mirrored by that of the meat industry where world production in the next two decades is expected to grow at a rate of 1.9% per annum, compared with 2.8% for the preceding two decades (FAO, 2000). In contrast, the aquaculture industry had a 5% growth rate in the period 1950 – 1969 and then increased to 8% until 1990, from where it has increased further to 10% per year (Figure 1.1). Current trends, therefore point to the increasing importance of aquaculture in the food producing sector.

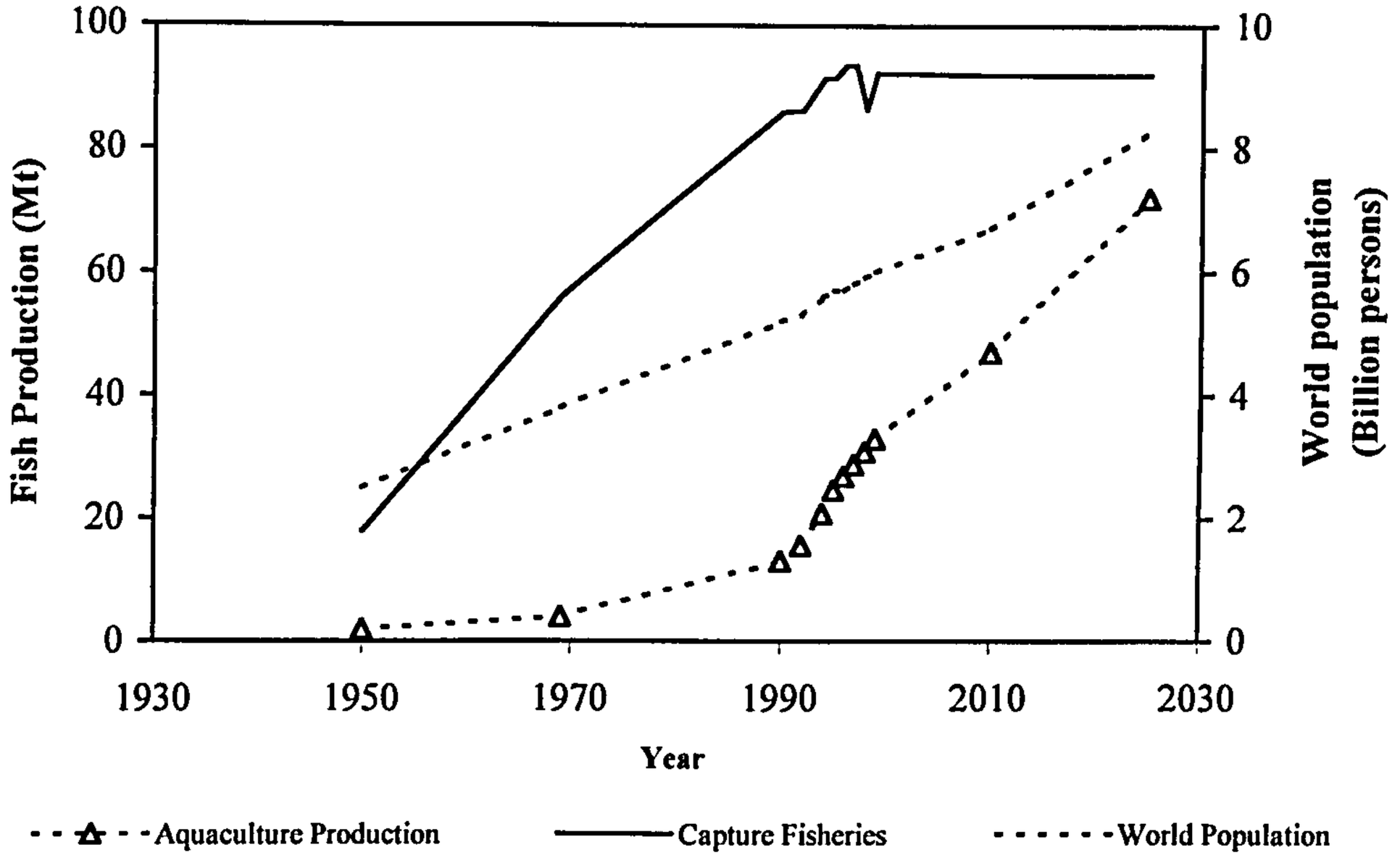


Figure 1.1. Real and projected growth of aquaculture industry, capture fisheries and world population.

1.1.1. World Aquaculture Production

An examination of global aquaculture production shows an increase in the importance of the industry. Aquaculture provided 26% of global fisheries production in 1998 worth an estimated 52.5 billion U.S. dollars with a total production of 30.9 million t and a further 8.5 million t of seaweed. In terms of contribution to food fish supplies (not considering produce converted to animal feeds), aquaculture contributed 31% in 1998, a rise from 19% in 1990.

Low income food deficit countries dominate the top aquaculture producing countries (Table 1.1). In 1998, Asian countries, in particular China, produced in excess of 27 million t (69% by weight of world production) worth an estimated value of 25.5 billion dollars (FAO, 2000). However, this was mostly the production of low value species such as seaweed (*Laminaria japonica*). In contrast, Japan contributed only 3.2% by weight to

global production but due to the culture of high value species such as oysters (*Crassostrea gigas*) this generated a greater market value in terms of weight produced (FAO, 2000).

Table 1.1. Major aquaculture producing countries and value of production in 1998 (FAO, 2000).

Country	Quantity* (X 10^3 t)	Value (US$ millions)	Value t^{-1} (US$ thousands t^{-1})
China	27,072	25,449	0.94
India	2,030	2,223	1.10
Japan	1,290	4,126	3.20
Philippines	955	639	0.67
Indonesia	814	2,150	2.64
Korea	797	766	0.96
Bangladesh	584	1,494	2.56
Thailand	570	1,807	3.17
Vietnam	538	1,357	2.52
Other	4,782	12,448	2.60
Total	**39,432**	**52,459**	**19.36**

Note: *including aquatic plants.

It has been estimated that more than 20,000 species of fish have been described with in excess of 100 of these being cultured for commercial purposes (Shepherd and Bromage, 1992). Although a more diverse activity in comparison with agriculture, the dominant aquaculture activity in 1998 was finfish culture accounting for 44% of the total world production (Table 1.2). Of the other remaining activities, crustaceans accounted for 4% while the production of molluscs and aquatic plants had 23 and 21% share of the total production respectively. Most aquaculture production, excluding the culture of aquatic plants, originated in freshwater (18.1 million t) with 10.8 and 1.9 million t produced in marine and brackish water environments respectively (FAO, 2000). Finfish culture, mainly carp (*Cyprinus carpio*), salmonids (*Salmo salar* and *Oncorhynchus mykiss*) and

tilapias (*Oreochromis* spp. and *Sarotherodon* spp.), accounted for 98% of freshwater productivity but only 8% in the marine environment, which was dominated by the production of molluscs (47%) (FAO, 2000).

Table 1.2. Global aquaculture production of major species groups in 1998 (FAO, 2000).

Species Group	Quantity ($\times 10^3$ t)	Value (US$ millions)	Value t^{-1} (US$ thousands t^{-1})
Freshwater fish	17,355	19,737	1.14
Molluscs	9,143	8,479	0.93
Aquatic plants	8,568	5,377	0.63
Diadromous fish	1,909	5,907	3.09
Crustaceans	1,564	9,234	5.90
Marine fish	781	3,396	4.35
Other aquatic animals	111	330	2.97
Total	**39,431**	**52,460**	**18.01**

Cage aquaculture is responsible for less than 1% of the total world aquaculture production (FAO, 1996). However, when examined on a regional basis, cage aquaculture may be considered to have a disproportionately large role (Beveridge, 1996). For example, in Scotland, of the total aquaculture production, cage cultivation represents approximately 91% of freshwater activity (including the culture of salmon smolts) and more than 99% production in the marine environment (SERAD, 2000). Similarly, Japan's yellowtail (*Seriola quinqueradiata*) and western Europe's Atlantic salmon (*S. salar*) industries are almost exclusively based on cage culture systems (Beveridge, 1996).

1.1.2. The Contribution of World Aquaculture to Global Fish Production

Today, the total global production of fish is an estimated 125 million t (*c.* 30 million t being reduced to fishmeal and oil), an increase of *c.* 20 million t since 1990. The aquaculture industry is credited with this increase in growth accounting for *c.* 32.9 million t of today's total global fish production (FAO, 2000).

The total annual human consumption of fish per caput has also increased from 9 kg in the 1960's to an estimated 15.4 kg in 1999, with a concurrent increase in world population, almost doubling in the same period. Due to a number of factors such as the growth in per caput income, diet diversification and the slow growth of the meat industry, it has been forecast that the global average per caput consumption of aquatic products could grow to 19 - 20 kg by 2030, raising total food use of fish to 150 - 160 million t (FAO, 2000). With diminishing conventional stocks, it has been suggested that aquaculture will expand further in order to satisfy the increasing demand for food fish and to compensate for the shortfall from capture fisheries (Beveridge, 1996; Welcomme, 1996; New, 1997, 1999; FAO, 2000, Naylor et al., 2000). World population is predicted to increase to 8.1 billion in 2030 (UN, 2000). The maximum sustainable yield of capture fisheries has been estimated at no more than 100 million t per year (FAO, 1999). Using the above predictions, it can be therefore estimated that the aquaculture industry will have to increase by *c.* 50 - 60 million t by 2030 in order to meet the world demand for food fish with increasing population.

1.1.3. Scottish Production

The total production of aquatic products through aquaculture in 1999 was 132,619 t in Scotland. This reflects a growing industry with a doubling of production occurring since 1990. The predominant species of production were *S. salar* and rainbow trout (*O. mykiss*) with 126,686 and 5,834 t being produced respectively. Cage culture was the main method of production, accounting for 57% of rainbow trout and more than 99% for Atlantic salmon (Table 1.3). Also reported was an increasing diversification of the Scottish aquaculture industry, with the production of alternative species such as Artic charr (*Salvelinus alpinus*), brown trout (*S. trutta*), cod (*Gadus morhua*) and halibut (*Hippoglossus hippoglossus*) accounting for *c.* 99 t of the total production. Projections for year 2000 suggest a 174% increase in the culture of alternative species, in particular *G. morhua* and *H. hippoglossus* (Table 1.4).

Table 1.3. Method of production and production tonnages of rainbow trout and Atlantic salmon in Scotland (SERAD, 2000).

Culture system	Environment	Rainbow trout (t)	Atlantic salmon (t)
Cage	Marine	1,075	126,492
	Freshwater	2,245	0
Tank and hatchery	Marine	3	194
	Freshwater	112	0
Pond and raceway	Marine	0	0
	Freshwater	2,399	0
Total		**5,834**	**126,686**

Table 1.4. Major species produced in the Scottish aquaculture industry, 1990 - 2000 (SERAD, 2000).

Species	1990 (t)	1999 (t)	2000* (t)
S. salar	32,351	126,686	130,837
O. mykiss	3,183	5,834	--
G. morhua	--	0.1	26
H. hippoglossus	--	3.6	57.5
S. alpinus	--	2.8	6
S. trutta	--	92	182.5

Note: * estimated production for 2000.

1.2. AQUACULTURE PRODUCTION SYSTEMS

1.2.1. Definition and Classification

The purpose of a rearing system is to hold the organisms captive while they increase in biomass, by minimising losses through predation and disease (Beveridge, 1996). The production systems used for aquaculture vary depending on the taxonomy group being cultured, life cycle of the species and nature of the environment (Figure 1.2). Selection of an appropriate production system for a given species will depend on existing socio-economic factors such as technology, human resources and available capital (Shepherd and Bromage, 1992). Aquaculture rearing techniques may be defined in terms of the intensity of the production (Pillay, 1992). This definition refers to the amount of biological material harvested per unit area (or volume) of the culture system, and also to the degree of manipulation of the natural process (Midlen and Redding, 1998). As with the nature of the culture system employed for a given species, the level of intensification may also be governed by existing local conditions. The culture system may be therefore classified on the basis of feed inputs as extensive, semi-intensive and intensive.

Extensive systems are characterised by unsophisticated technology, low stocking densities and low harvest per unit area of culture system. They also rely solely on available natural foods. For example, the growth of blue green algae is promoted by the addition of fertiliser to pond culture systems providing an ample source of food for the culture of milkfish (*Chanos chanos*) in Indonesia (Midlen and Redding, 1998). Similarly, semi-intensive culture relies on natural food sources for biomass growth but in addition, fish are provided with low grade feedstuffs to supplement the intake of natural food. For example, the semi-intensive cage culture of silver (*Hypophthalmichthys molitrix*) and common (*Cyprinus carpio*) carp in tropical freshwaters fed a combination of materials including rice bran and domestic wastes (Beveridge, 1996). Extensive and semi-intensive culture is widely practiced in developing countries, as in much of Southeast Asia.

In contrast, in many developed countries, aquaculture production systems have moved towards more intensive culture systems (*e.g.* the cage culture of *S. salar* in the marine environment). These production systems are characterised by a food supply which is almost entirely controlled in the form of high grade artificial feed composed of fishmeal and other food sources. Intensive culture systems achieve more output from a given production unit through the application of greater levels of technology, with the use of specially designed rearing facilities for each stage of the production cycle. One expression of this level of technological intervention is that a high rate of water turnover is often required to maintain water quality within the system (Pillay, 1992; Beveridge, 1996; FAO, 1999).

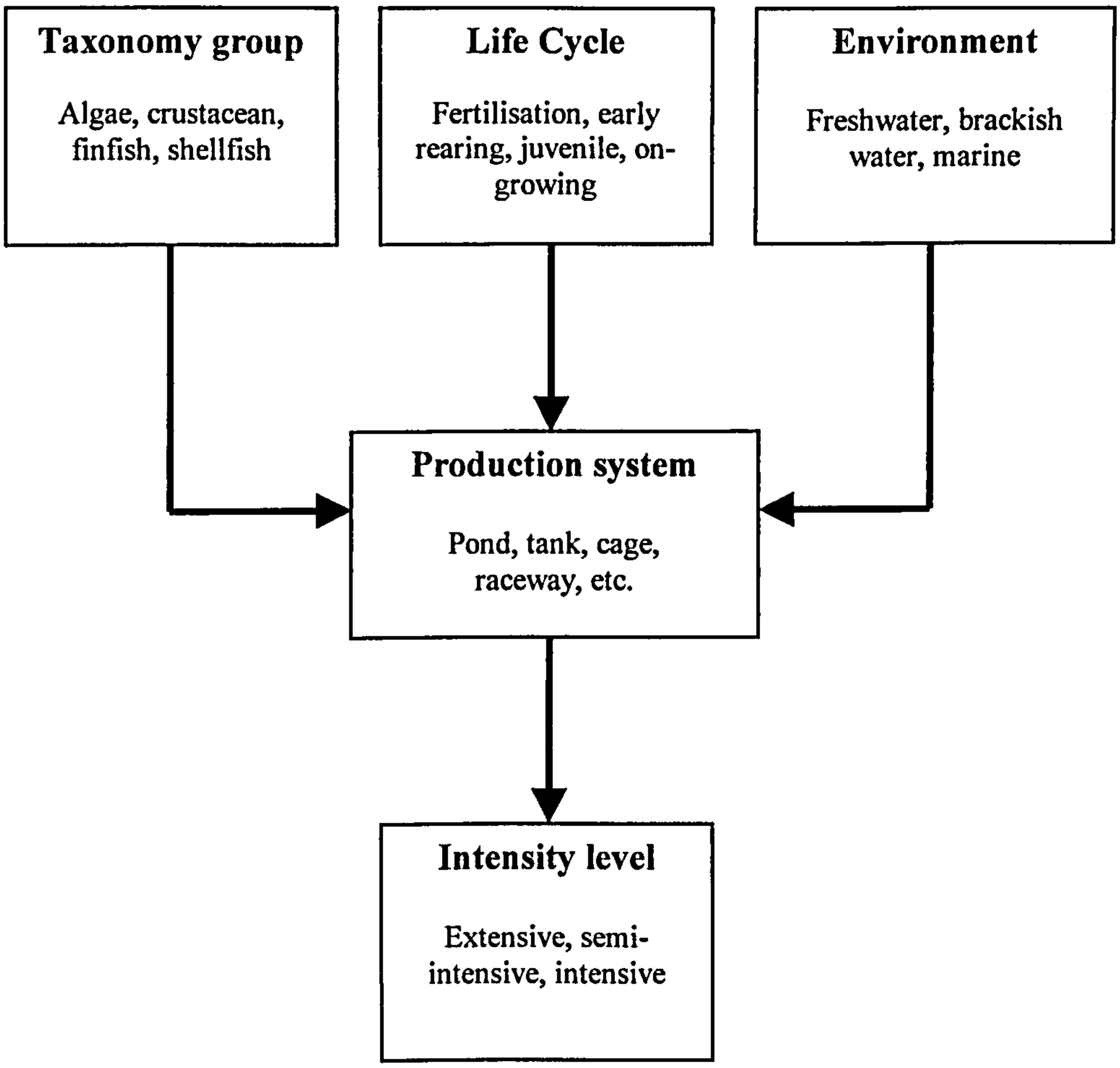


Figure 1.2. Characterisation of aquaculture production systems (Elberizon, 2000).

1.2.2. Cage Systems

An aquatic cage may be defined as a volume enclosed with some type of mesh surrounding its sides and bottom, forming a container for aquatic animals (Huguenin, 1997). Cage aquaculture has been used for centuries in all kinds of environments including freshwater, estuarine and seawater. There is an enormous diversity in the type and design of cages (Table 1.5) and they may be classified in a number of ways including their basis of operation and on their means of structural support. There are therefore four basic types of cages namely fixed, floating, submersible and submerged. By far the most widely used is the floating cage (Beveridge, 1996) which comprises a floating collar

(square, rectangular octagonal or circular) from which a flexible bag usually made of synthetic netting hangs (Figure 1.3). Cage culture systems vary in size from a few metres to several hundred metres squared. In most cases the farm site will consist of many cages moored together.

Table 1.5. Classification of cage systems (Huguenin, 1997).

Where operated	Surface Submerged Maine, estuarine, freshwater
Means of support	Fixed to bottom (via pilings) Floating
Type of structure	Rigid (structure and mesh) Flexible (mesh only)
Service access	Catwalk Boat only (no catwalk)
Operating parameters	Biomass loading (extensive/intensive) Species
Environmental conditions	Sheltered/exposed/open water

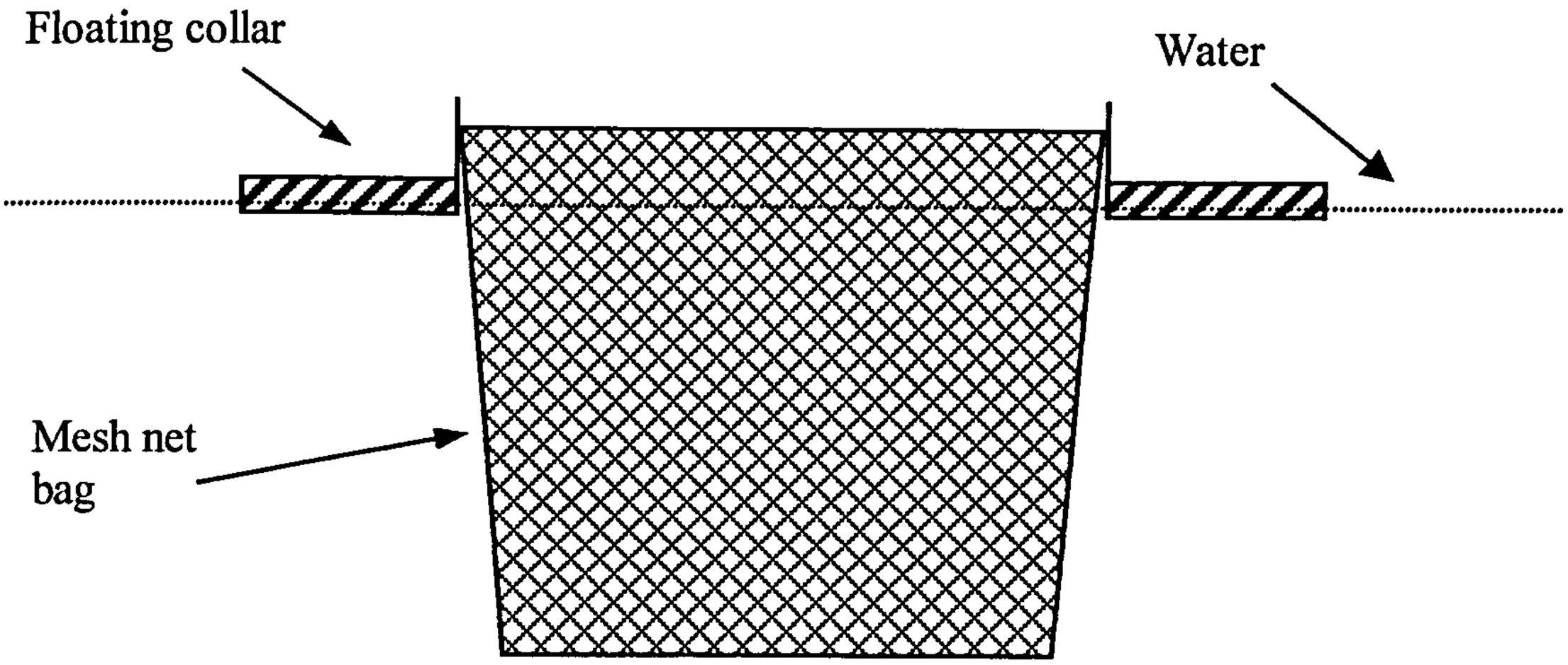


Figure 1.3. Schematic of a floating net cage.

1.3. ENVIRONMENTAL IMPACTS OF AQUACULTURE

1.3.1. Aquaculture and the Environment

As with any production process, there will be the creation of unwanted by-products which may often have little or no economic value. Aquaculture is no exception, with the production of fish also leading to the production of wastes (Figure 1.4). The potential impact of aquaculture wastes on the environment in terms of waste loadings is small in comparison with other sources of pollution (Table 1.6), however the characteristics of the environment (*Section 1.3.3*) into which they are released ultimately determines their impact. Waste discharge from an aquaculture production unit represents a localised, point source discharge into unpolluted waters and thus the impact could be significant (Gowen et al., 1990).

This is of particular concern in relation to cage culture systems. These systems, unlike land based systems, depend directly on the immediate physical environment for water and oxygen supplies to maintain production and the elimination of wastes. Waste products are

discharged directly to the surrounding water environment with no intermediate stage and thus have a greater potential for impact on the environment than land based systems.

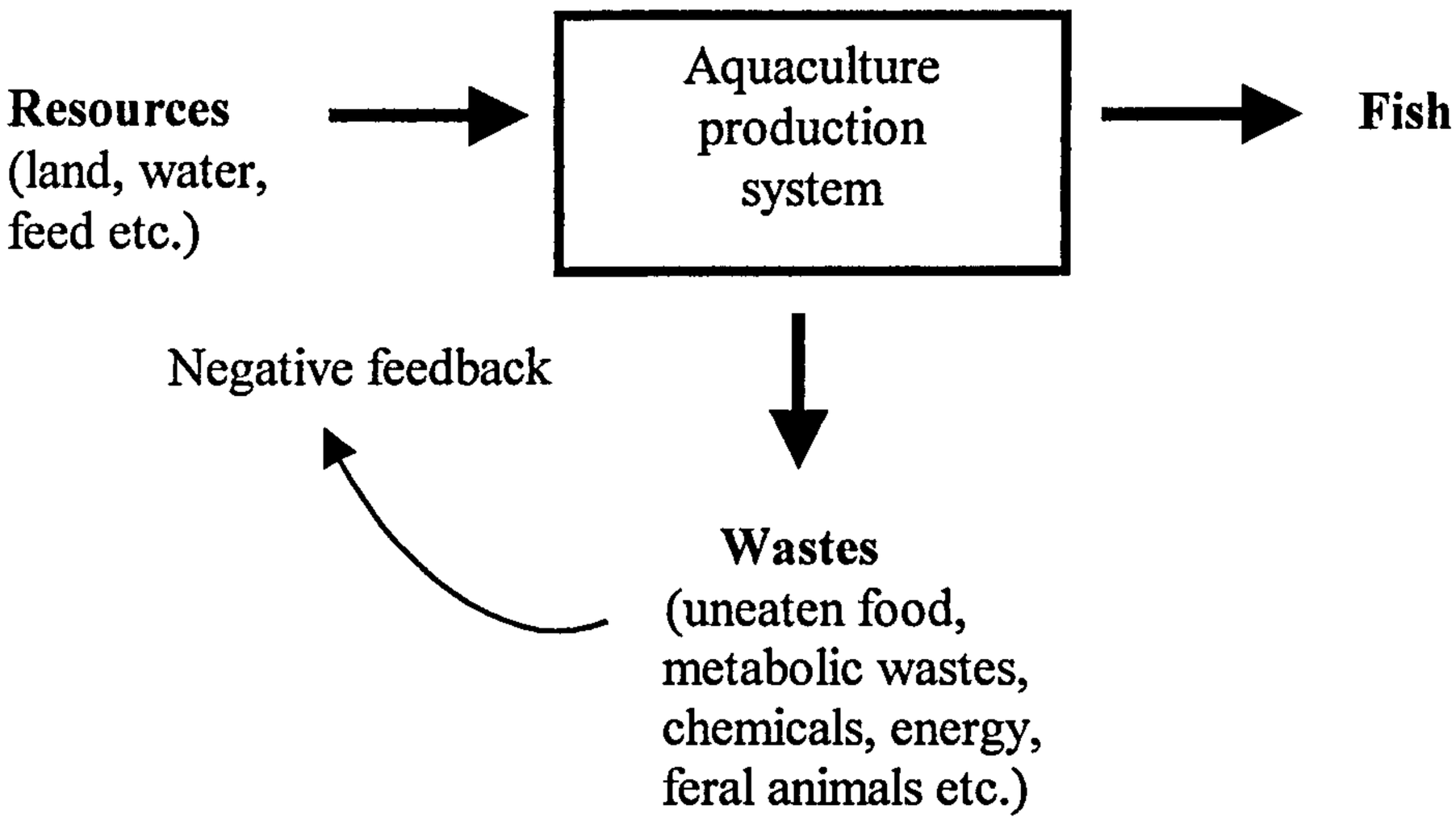


Figure 1.4. The main inputs and outputs of fish farming (Beveridge et al., 1997).

Table 1.6. Biological oxygen demand (BOD), total nitrogen (N) and total phosphorus (TP) concentrations (kg m^{-3}) of various organic wastes (NCC, 1990; Sherwood, 1993; [a]Kelly et al., 1997; Midlen and Redding[b], 1998; SEPA, 1997[c]).

Waste	BOD	N	TP
Silage effluent	65,000	2,700	560
Pig slurry	25,000	4,000	1,400
Cattle slurry	17,000	4,000	700
Untreated sewage	400	55	15
Tank fish farming	0.0 – 88[a]	0.5 – 4[b]	0.005 – 1.141[a]
Excellent standard water [c]	≤0.025[c]	≤0.01	≤0.004[c]

Note: Excellent standard water based on Scottish river classification scheme as used by SEPA.

In recent years the industry has attracted concerns regarding its environmental impact. The rapid expansion of shrimp farming in Asia and Latin America through unsuitable site selection procedures caused considerable environmental and often social damage (FAO, 1999).

Environmental impacts of aquaculture are not restricted to the direct impacts on the immediate physical environment as a result of negative inputs and outputs. Fishmeal for food pellet production, for example, is often obtained at a distance from the production area. Folke and Kautsky (1992) estimated that 15% of the capture fisheries from the North Sea was required to support the production of 80,000 t of farmed Atlantic salmon in Norway. Aquaculture is reliant on a wide range of natural resources for the construction of aquaculture systems and ancillary facilities such as roads for farm access. The use of mechanised farm equipment can cause a disturbance to wildlife as well as local communities (Pillay, 1992; Buschmann et al., 1996; Beveridge, et al., 1997). Also, there is often the competition for water resources with recreational facilities and the diminishing of scenic waterfronts with the introduction of intensive culture systems (Pillay, 1992).

Conversely, aquaculture can contribute positively to society creating economic incentives for local communities with the promotion of tourism and sport fishing, and nationally with the export of fish. Aquaculture can also be beneficial to the environment. Integrated aquaculture production can be used in the recycling of nutrients and organic matter (Buschmann et al., 1996; Troell and Berg, 1997). Inland pond culture can enhance habitat and landscape diversity within agro-ecosystems and in highly integrated systems, fish ponds play a vital role in supporting other activities and in water conservation. Wastes are, in general, confined to the pond at all times and can often be readily utilised in the production of other crops (Beveridge et al, 1997).

It should also be noted that the culture of molluscs and seaweeds has very little impact on the aquatic environment (FAO, 1999). Similarly, the same is true for most forms of carp culture and these cultures account for more than half of the total global aquaculture production (*Section 1.1.1*). It is the move towards intensification and in particular the employment of water based systems such as intensive cage culture that the negative impacts of aquaculture are mostly associated.

1.3.2. Cage Aquaculture wastes

Wastes from aquaculture include all materials used in the process which are not removed from the system during harvesting (Cripps et al., 2000). This can include dead and escaped fish, pathogens, chemicals and therapeutics (Ackefors and Enell, 1994; Beveridge, 1996; Bergheim and Åsgaard, 1996; Cripps et al., 2000). However, the most significant source of potential pollution is feed derived aquaculture wastes such as uneaten feed, undigested feed residues and excretion products (Persson, 1991; Pillay, 1992; Cripps and Bergheim, 2000). Fish reared in intensive cages are fed, through hand or automated feeding systems, artificial food in the form of feed pellets (Beveridge, 1996). Of the feed presented to the fish stock, a proportion is not eaten. Unconsumed feed may be a result of poor appetite, undetection, rejection due to improper taste or simply because it has been washed away from the cage by water currents (Smith et al., 1995; Ang and Petrell, 1998). It has been estimated for intensive marine cage production of Atlantic salmon, between 1 – 15% of feed fed is wasted in this fashion (Juell, 1991; Ang and Petrell, 1998). Of the feed eaten by the fish, a proportion is indigestible and is excreted as solid faecal waste. The metabolic breakdown of eaten digestible feed will result in the excretion of dissolved waste products, such as ammonia-nitrogen (NH_4-N),

carbon dioxide (CO_2) and excess nutrients, through the gills and in urine (Shepherd and Bromage, 1992; Beveridge, 1996).

A wide range of chemicals are used in the salmonid aquaculture industry, from antifoulants used on cage nets to pigments in feed pellets. With the intensification of the industry and the resulting increase in the risk of disease transmission, the vast majority of chemicals are used for the treatment or prevention of disease (Pillay, 1992; Beveridge, 1996). Therapeutants may be grouped in five main categories; antibacterials or antimicrobials, antiparasitics, antifoulants, anaesthetics and disinfectants (SNIFFER, 1998). They may be administered orally as feed additives, immersion of the stock in a chemical solution or mass injection of the stock. Because of the unconfined nature of the cage culture system, all chemotherapeutants except those assimilated by the cultured fish themselves are released to the surrounding environment, either by direct release as a result of immersion therapy or from urine, faeces and uneaten feed. Most therapeutants, however, are not of great concern to the freshwater phase of salmonid production. For example antifoulants are used to control the growth of organisms on tanks and nets, but fouling is much less of a problem in freshwater than in the marine environment and cage farmers either use untreated or bitumen treated nets (NCC, 1990).

1.3.2.1. Waste loadings

The quantity of feed derived waste will depend on the mass of feed added to the culture system and the efficiency of conversion of feed by the fish. Furthermore, waste loadings are due directly to the type of culture employed and the species of fish cultured (Pillay, 1990). The feed conversion ratio (FCR) is the ratio of the gain in the wet body weight of the fish to the amount of feed fed. The true FCR includes wasted feed and mortalities. Feed conversion ratios of less than 1 : 1 are possible with commercial diets, as the pellet

being fed is a "dry" diet, and a high percentage of weight gained by the fish, is water trapped in the tissues and cells. Feed conversion ratios with commercial "dry" diets are typically in the region of 1 : 0.8 to 1 : 1.5 (AquaText, 2000), but are highly dependent on the culture system employed. The higher wastage rates observed in salmonid cage culture result in the use of 1.3 – 2.0 kg of feed to produce 1 kg of fish.

The general method of deducing losses from cage culture is through adopting a mass balance calculation approach (Persson, 1991). It is based on the principle that the amount of energy, in the form of solid feed, entering the system must be equal to the amount of energy leaving the system, in the form of waste material and excretory products and transformation to biomass (Cho et al., 1991). Uneaten feed, faecal material and excretory losses may be estimated through a combination of field and laboratory data, and knowledge of feed quantity and composition, FCR's, digestibilities and faecal composition (Nijhof, 1994; Beveridge, 1996).

For example, the typical N and phosphorus (P) content of a food pellet is *c.* 6 - 8% and 1 – 1.5% respectively, whilst a fish carcass is composed of *c.* 2.5 – 2.7% N (Wallin and Håkanson, 1991) and 0.4 – 0.5% P (Weston et al., 1996). Taking this into consideration and assuming a typical cage culture FCR of 1.3, it may be seen that a substantial proportion of N and P therefore leaves the culture system as waste, *c.* 75 - 101 kg N and 10 – 20 kg P for every tonne of fish harvested.

This approach however does not take into consideration the effect of the environment on waste material and the nature of the waste material in terms of its chemical form (organic or inorganic) or physical state (solid of dissolved). Furthermore, the bioavailabilty of the waste material and the contribution of other waste sources are not taken into account (Kelly, 1995; Beveridge et al., 1997). Whilst it has obvious deficiencies, the mass balance

approach does give an indication of the quantity of waste produced and has been used in a variety of investigations relating to the loss of nutrients carbon (C), N and P from cages (Table 1.7). Estimates will vary between studies due in part to differences and improvements in husbandry practices (Gowen, 1991) and hydrodynamic conditions of the cage culture site.

Table 1.7. Mass–balance studies of C, N, and P fluxes in salmonid cage aquaculture.

Nutrient added as feed (100%)	Retained (%)	Dissolved (%)	Sedimented (%)	Environment	Reference
Carbon	28	65	7	Fresh	Penczak et al., 1982
	23	69	8	Marine	Gowen et al., 1988
	21 - 22	11 - 58	25 - 66	Marine	Hall et al., 1990
	10.7	48.6	40.7	Fresh	Elberizon, 2000
Nitrogen	35	38	27	Fresh	Penczak et al., 1982
	27 - 28	48	23	Marine	Hall et al., 1992
	9.3	56.3	34.4	Fresh	Elberizon, 2000
Phosphorus	33	45	22	Fresh	Penczak et al., 1982
	17 - 19	25 - 30	50 - 57	Marine	Holby and Hall, 1991
	8 - 18	45 - 53	36 - 38	Fresh	Troell and Berg, 1997

It is evident that a large proportion of waste from the culture process is in dissolved form.

Particulate wastes, however, may also contribute to the dissolved phase through the processes of leaching and dissolution (Figure 1.5). Elberizon (2000) estimated that in freshwater the degree of leaching of C and N from faeces at a fall velocity of 0.03 m s^{-1} and depth of 9 m (also the depth of collection system) was 3.7 and 15.1% respectively. In the same study, over a period of 15 days at a temperature of 10°C, dissolution of C and N

was estimated at 12.1 and 48.0% respectively. Similarly, Garcia-Ruiz and Hall (1996) and Behmer et al. (1993) estimated ranges of 11.1 – 64% for leaching of P after 24 h. Kelly (1993) estimated the release of P for sediments underneath cages at a rate of 1.5 – 57.6 mg P m^{-2} d^{-1}, with the variation in release rates associated with the intensity of cage farming on the sampled sites.

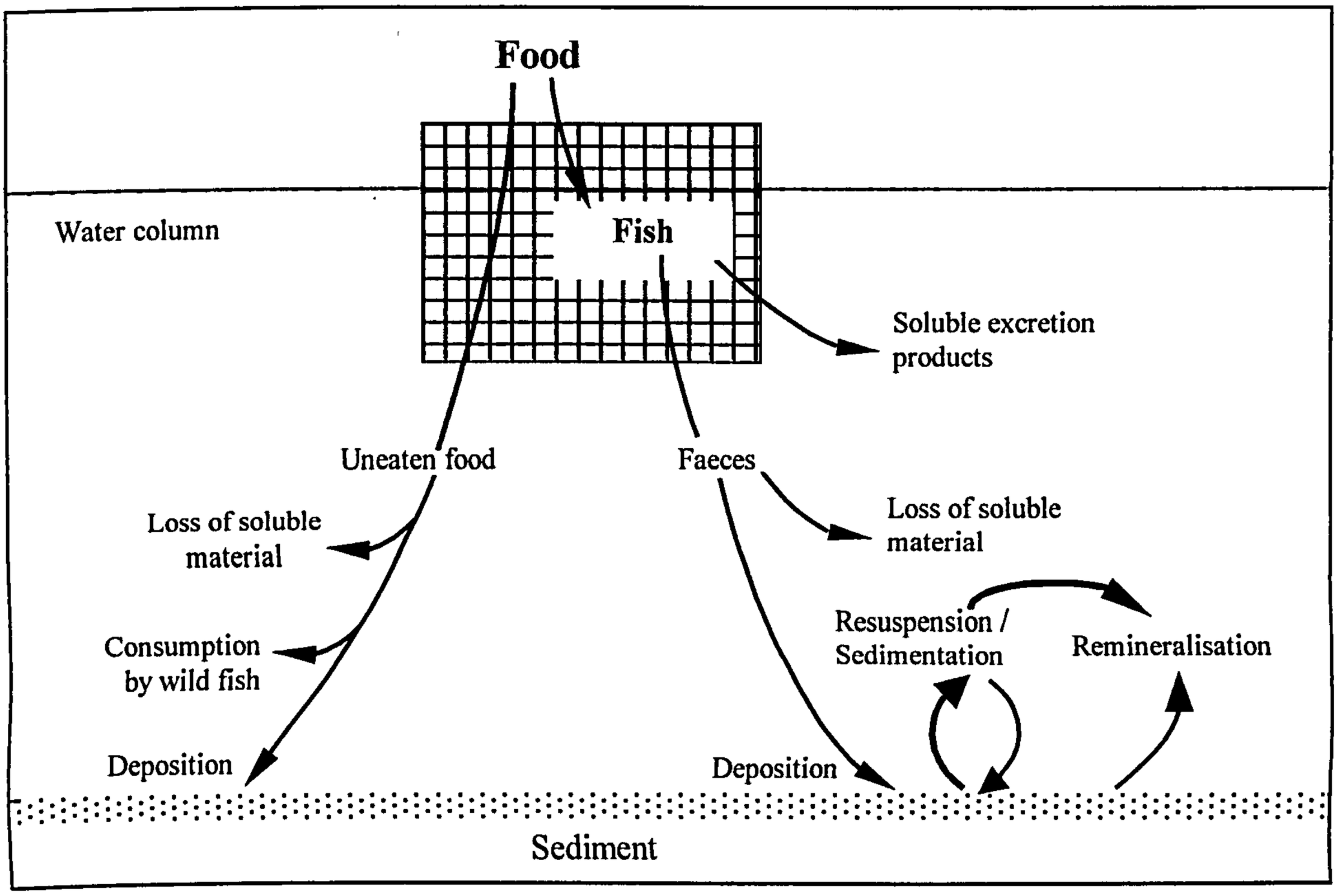


Figure 1.5. The fate of material from intensive cage aquaculture (Gowen et al., 1990).

1.3.3. Impact of Cage Aquaculture Wastes on the Environment

The magnitude of the negative effects of aquaculture wastes on the environment will depend on the carrying capacity of the receiving environment. Catton (1986) defined an environment's carrying capacity as its maximum persistently supportable load. Factors

contributing to the ability of the environment to absorb negative inputs of aquaculture wastes include a number of physical and biological factors.

Physical characteristics of the receiving water body such as the rate of dilution of the waste are critical as is the nature of the waste which will also determine the extent of impact of aquaculture on the environment. Other important factors are the method of feeding and the composition of the feed itself (Ackefors and Enell, 1994). The intensity and size of production system will also be a factor.

In general, the recipient of particulate organic waste from fish cages is the sediment, and the recipient of soluble waste is the water column, although there may be an exchange of material between the sediment and the overlying water body (Gowen, 1990). Uneaten feed and faeces will result in the leaching of nutrients, as they fall through the water column and lie on the sediment floor, with the subsequent enrichment of the overlying water column. The potential impacts of particulate and soluble wastes on the environment are discussed below.

1.3.3.1. Nutrient enrichment

The increase in concentration of any dissolved nutrient is termed hypernutrification and the resultant increase in primary productivity because of this is known as eutrophication. The dissolved nutrients of most concern are P and N, in their various chemical forms. These are important nutrients for plant growth and their increase in natural waters can significantly affect the balance of natural ecosystems. In freshwaters, P is of most concern as it is the limiting growth factor for aquatic plants in most regions while N limits the process in marine waters. Hypernutrification can result in an increase in phytoplankton growth and a change in the species composition of phytoplankton (Gowen et al., 1990). In

cases of extreme nutrient enrichment, an increase in the growth of cyanobacteria and the domination of zooplankton by *Daphnia* spp. have been observed indicating a move towards eutrophication of the water body (Philips, 1985; Gowen et al., 1990; NCC, 1990; Cornel and Whoriskey, 1993; Axler et al., 1996; Baffico and Pedrozo, 1996). Due to lower dilution rates and water movement, nutrient enrichment resulting from aquaculture is more frequently observed in freshwater than marine environments. Although it is feasible for cage aquaculture to occur without the enhancement of water column productivity (Philips, 1985), many studies have shown its contribution to nutrient enrichment in both fresh and marine waters. Seasonal variations of ammonia concentrations were measured in surface water in the vicinity of a Norwegian marine cage farm and were found to be 2 - 10 times greater than background levels with optimum concentrations occurring in autumn (Ervik et al., 1985). The presence of cage farms in freshwater have been shown to increase P levels in the water column (Penczak et al., 1982; Enell and Lof, 1983; Costa-Pierce, 1996). Yokom et al. (1997) reported a ten fold increase in TP during intensive salmonid production in a mine pit lake with recovery occurring after the termination of aquaculture production. Furthermore, nutrient enrichment in a freshwater lake in Quebec, Canada, was found to vary seasonally (Cornel and Whoriskey, 1993).

1.3.3.2. Sediment and benthic community

Attention is increasingly turning to the effects of solid waste from cages on the environment. Solid wastes will settle to the sediment beneath the cages and cause enrichment of the benthic ecosystem. The deposition of these particulate organic wastes has a major impact on the chemistry of the sediment and the organisms that live within it (Gowen et al., 1990). High net depositional rates can cause an accumulation of organic detritus in the sediment which can overwhelm the feeding capacity of the benthos and

result in the formation of bacterial mats (Silvert, 1992). As a result, sediment oxygen consumption is elevated and there is a decrease in the redox potential (E_h) of the sediment leading to the development of anoxic ($E_h < +100$) and possibly anaerobic ($E_h < -100$) conditions. In turn, the anaerobic microbial degradation of organic material leads to the generation of hydrogen sulphide (H_2S) and methane (CH_4) from the sediment to the overlying water column (Enell and Lof, 1983; Hall and Holby, 1986; Gowen et al., 1990; Gowen et al., 1991; Kelly, 1992; Silvert, 1992; Cornel and Whoriskey, 1993; Hargrave et al., 1993; Weston et al., 1996). The production of H_2S gas is of concern, particularly in the marine environment due to the greater availability of sulphates in comparison with freshwater, as it is highly toxic to fish and has been reported as the cause of gill damage (Gowen et al., 1991).

The resultant change in sediment chemistry will cause an alteration in the composition of macrobenthic communities. There is, in general, a positive correlation between organic material accumulation and biomass of macrobenthos while a negative correlation exists between organic matter accumulation and species diversity. In areas where there is a high degree of organic enrichment, an abundance of pollution tolerant species such as chironomid larvae and polychaetes, which are either absent or in low densities in surrounding undisturbed sediments, will occur (Gowen, 1991; Cornel and Whoriskey, 1993; Beveridge, 1996; Loch et al., 1996; Hargrave et al., 1997). Furthermore, the presence of macroinvertebrate groups sensitive to water quality such as Ephemeroptera decrease, if present at all (Beveridge, 1996; Loch et al., 1996).

In addition to the change in composition of benthos and generation of gases from anaerobic degradation of organic material, settled particulate wastes can cause nutrient enrichment. Uneaten feed and faecal matter from intensive fish farms are unlikely to have have much higher levels of P and N than allochthonous sediment, thus increasing

concentrations where they are deposited. The mineralisation of nutrients from organic wastes can thus result in the additional nutrification of the water column (Wisniewski and Planter, 1987; Kelly, 1992; 1993). N is released from enriched sediments in the form of dissolved organic compounds and ammonium (Gowen et al., 1991; Hargrave et al., 1993; Weston et al., 1996; Troell and Berg, 1997). This is of particular importance for P, as unlike N, it is mainly associated with the solid fraction of organic waste (Merican and Philips, 1985; NCC, 1990; Bergheim et al., 1993; Kelly et al., 1996). The promotion of anaerobic conditions within the sediment can enhance the flux of P from the sediment as soluble phosphate species (Enell and Lof, 1983; Holby and Hall, 1991; Kelly, 1992; Troell and Berg, 1997).

The extent of impact of organic wastes, as with soluble wastes, will largely depend on a number of factors as outlined in *Section 1.3.1*. The area of benthos affected by organic waste deposition is generally very localised with typical measurable benthos changes at distances of 15 – 50 m from the farm perimeter (Gowen, 1988; NCC, 1990). These studies, however, have been limited mainly to the marine environment and some have reported impacts at greater distances of up to 250 m (Weston, 1991; Johannessen et al., 1994). This may often be attributed to poor management or unexpected hydrographic conditions (Beveridge, 1996). The impact of organic wastes on freshwater benthos should exhibit the same general structural and functional changes as their marine counterparts (NCC, 1990; Cornel and Whoriskey, 1993).

1.3.3.3. Chemicals

There is particular concern regarding the use of chemotherapeutants due to the large quantities discharged and the limited knowledge of their effects on the aquatic environment (Beveridge, 1996). Studies regarding the impact of chemicals on the

environment have been limited mostly to the marine environment and literature on the freshwater effects is scarce. Of the many concerns includes the persistence of antibiotic residues in the environment and their accumulation in sediments (Samuelsen, 1989; Stoffregen et al., 1996). This may in turn have an adverse impact on the microbial community affecting their ability to degrade organic waste (Kupka-Hansen et al., 1991) and also increase the antibiotic resistance of pathogens (Kerry et al., 1994; Stoffregen, 1996). Another concern over the use of therapeutants is the impact on aquaculture itself. The regular use to prevent the outbreak of infection as opposed to their use as a response to infection may lead to the development of resistant strains of bacterial diseases (Midlen and Redding, 1998).

1.3.3.4. Other impacts of cage aquaculture

The impact of aquaculture on the environment is not just attributed to the production of food derived waste materials and chemicals. The introduction of foreign species and the movement of fish has been reported to promote disease transmission (Ridell, 1993; Pursell et al., 1996). Furthermore, the introduction of new species and the escape of farmed stock can spread disease to wild populations and reduce aquatic biodiversity through habitat modification, competition and interbreeding with native stocks (Beveridge, 1996; Beardmore et al., 1997; Angel et al., 1998).

Chapter 2

Waste Management in Aquaculture

2.1. INTRODUCTION

Aquaculture, unlike some process industries, is highly reliant on a clean environment in which to operate. It is therefore in the interests of aquaculturalists to ensure a clean, safe environment, at least in the vicinity of their farm (Cripps, 1991). Furthermore, the environment is an issue of growing importance with consumers throughout the world increasingly unwilling to purchase food products without regard to how it is produced (Rackham, 1995). The development of sustainable aquaculture is therefore critical for the environment and the industry itself. Sustainable development has been defined by the FAO (1991) as:

> *".....the management and conservation of the natural resource base, and the orientation of technological and institutional change in such a manner as to ensure the attainment and continued satisfaction of human needs for present and future generations. Such sustainable development conserves land, water, plant and animal genetic resources, is environmentally non-degrading, technically appropriate, economically viable and socially acceptable."*

With the increase in the trend of global aquaculture production, it is of utmost importance for the industry to become socially acceptable and demonstrate its ability not to jeopardise the aquatic environment it uses. This is particularly important in relation to intensive cage culture in the freshwater environment where the negative impacts of aquaculture are most observed. The goal of environmental sustainability implies a move

away from monoculture intensification. Although this is unlikely to happen in the near future, the industry can develop and utilise existing technologies and methodologies to reduce the impact it may have on the environment.

2.2. REDUCING THE IMPACTS OF CAGE AQUACULTURE

Waste management can be often thought of simply as the end of pipe treatment of wastes from a production process. However, the most appropriate means to reduce the environmental impact of waste is to generate less waste in the first instance and ensure any waste discharge has a minimal effect on the environment. Reducing the impacts of cage aquaculture wastes on the receiving environment may be achieved through effective planning, waste minimisation techniques, and subsequently management of discharged wastes.

2.2.1. Effective Planning

A fundamental step in minimising the environmental effects of aquaculture is effective planning through appropriate site selection. Traditionally, this has been done with most emphasis placed on the suitability of the site for fish culture. Important site characteristics for fish culture include water quality, hydrodynamic considerations for dissolved oxygen replenishment, dispersion of metabolic wastes from the cage itself and most of all the suitability for establishing a profitable business (Beveridge, 1996). It is, however, apparent that certain sites or areas are particularly ecologically sensitive to cage aquaculture developments. Evaluation of site vulnerability is therefore a necessary step and is often required by law in some countries. In Scotland, presently, fish cage operators

are only able to discharge to a loch by obtaining a licence ("Consent to Discharge") from the Scottish Environment Protection Agency (SEPA). As exact discharges from cages are difficult to determine, licensing is typically based on a predetermined upper limit of annual biomass production. This biomass value is set with reference to established estimates of waste production (*e.g.* 200 - 500 kg solid waste and 8 - 12 kg of P per tonne of fish harvested), and the carrying capacity of the loch (*Section 1.3.3*).

Methodologies or tools for carrying out the environmental impact assessment (EIA) of a potential cage site and evaluating the carrying capacity of the environment include the use of predictive models. Modelling has been used to determine the impact of nutrient loadings on both the freshwater (Chapra and Tarapchak, 1976; Canfield and Bachmann, 1981; Nunberg, 1984; Kelly, 1995) and marine (Aure and Stigebrandt, 1990; Hakanson and Wallin, 1991; Gowen, 1994) environments. Similarly, models have been developed to predict the dispersion of organic waste from marine cages (Gowen and Bradbury, 1987; Gowen et al., 1988; Silvert and Sowles, 1996; Panchang et al., 1997) and more recently from freshwater cage production of salmonids (Elberizon, 2000). As a means for determining the carry capacity, modelling will require knowledge of the physical characteristics of the receiving water environment such as bathymetry and rate of dispersion (*Section 1.3.3*). Continuous monitoring of the environment will be required to ensure sufficient assimilation of waste discharges occurs (*e.g.* monitoring of DO levels, nutrient concentrations and species diversity).

2.2.2. Waste Minimisation

2.2.2.1. Feed composition

Fish feed in order to meet their energy requirements (Thorpe and Cho, 1995). An increase in the digestibility and bio-availability of proteins and nutrients will reduce the quantity of faecal solids produced. This has been achieved through the development of extruded feeds (as opposed to the more traditional pellet feeds) which have had undigestible complex polysaccharides such as starch gelatinised to produce more digestible carbohydrates thus reducing faecal waste production. (Seymour and Bergheim, 1991; Enell, 1995; Mayer and McLean, 1995; Thorpe and Cho, 1995; Cripps and Bergheim, 2000).

The discharge of N and P is also directly associated with the nutrient content in the feed. The increase in fat content from *c.* 20% to 30% and the associated protein reduction to about 40% (Kolsäter, 1995) has resulted in the reduction of N (as ammonia) loading from the aquaculture process by as much as 35% (Johnsen et al., 1993). Altering the source of protein in the feeds may also reduce the discharge of P. The dietary P requirement for optimum growth in fish is 0.5 – 0.8% (Enell, 1995). Animal protein sources, as used in fishmeal, have a high P content (1.5 – 3.2%) with the consequence that the majority is excreted. Substituting feed components of animal origin with plant derived components which contain less P has been shown to reduce P effluent loadings (Mayer and McLean, 1995). However, the bio-availability of P from plant sources to certain species of cultured fish is limited, though this may be overcome by the addition of P hydrolysing enzymes, such as phytases to the diet (Kolsäter, 1995; Mayer and McLean, 1995; Hardy, 1999).

2.2.2.2. Physical characteristics of feed

The physical characteristics of feed are also important in terms of waste reduction. The use of dry pellets instead of wet pellets significantly reduces the amount of waste (Alabaster, 1982). Hardness, colour and disintegration rate of feed are also important as this affects palatability to fish. Unstable pellets will increase the wastage rate if they disintegrate before being consumed (Smith et al., 1995; Weston et al., 1996). The use of extruded feeds is reported to improve stability, with pellets remaining 84% intact after 24 h in water as opposed to pressed pelleted feed which showed a 50% breakdown in less than 1 h (Mayer and McLean, 1995).

2.2.2.3. Feeding behaviour of fish

Knowledge of the feeding behaviour of the cultured species, together with appropriate feeding strategies and technology for feed delivery can also reduce the waste loading. Thorpe et al. (1990) demonstrated the importance of efficient scattering of feed over the entire cage area. Feed wastage was estimated at 1.4% of total food fed to the cage when feed was distributed evenly throughout the cage area in comparison 40.5% wastage as a result of feed delivery to a single point within the cage (Thorpe et al., 1990). In essence, better feed utilisation may be achieved by coordinating feed supply with patterns of feed intake (Thorpe et al., 1990; Kadri et al., 1991; Seymour and Bergheim, 1991).

Improvements in feeding technology have resulted in the development of automated feeders more capable of optimising feed utilisation in conjunction with maximal appetite. Systems have been developed in Norway (Bjordal et al., 1993) and Australia (Blyth et al., 1993) in which the automatic feeder is controlled by a hydroacoustic sensor. A sensor detects wasted pellets passing through the cage and when this reaches a pre-set level (i.e.

a predefined number of pellets), the automatic feeder is switched off. A modification of this system, known as the "Cage-Eye" system monitors the movement and distribution of the fish within the cage. As they rise to the surface, feeding is commenced and as they descend the automatic feeder is switched off. Automatic feeding systems can also aid in the reduction of chemical waste loadings for feed based therapeutants (SNIFFER, 1998).

2.2.3. Management of Discharged Wastes

While this is relatively easy to achieve in enclosed systems such as land based farms, the aim of reducing the impact of waste produced from intensive water based culture systems is more complex. Reduction of waste loadings are of particular importance in the freshwater environment where dispersion and dilution of waste materials is not as efficient as in marine waters. This is due to lower water current velocities in freshwater which are primarily wind induced as opposed to the marine environment which are governed mainly by tidal movements and the Coriolis effect (Horne and Goldman, 1994; Lewis, 1997). The options for the reduction of wastes discharged via cage culture are limited. Substrate vacuuming has been attempted (Rosenthal and Rangeley, 1989) but this only serves to move the waste from one place to another. It may also have an adverse impact on the ecology of the sediment (Weston et al., 1996). Similarly, submersible mixers have been deployed to prevent the accumulation of organic matter beneath the cages but this may result in the resuspension of sedimented material and subsequently reduce the water quality around the cages (Pillay, 1992). Preferably, effluent from the cage process should be intercepted before a detrimental impact on the aquatic ecosystem can occur. This would therefore require the need for reduction in waste discharge at the point of discharge.

2.3. SUBCAGE WASTE COLLECTION

There have been many attempts to enclose the floating cage (marine environment) using flexible impermeable plastic to form a bag pen. (Fast, 1991; Solaas et al., 1993). More recently, systems using a floating fabric bag enclosing the existing cage pen have been used to address waste issues (e.g. SEA System™, Future SEA Technologies Inc). The enclosed cage is supplied with pumped water allowing for the control of water quality and current speed. Particulate wastes are extracted by means of a waste trap concentrator, and removal efficiencies reportedly exceed 90% (Future SEA Technologies Inc., 1998; Fish Farming International, 1999). Although enclosure of the cage can enhance environmental performance of cage aquaculture, the requirement for filtration and pumping of water will result in the increase of operational costs to the fish farmer. Therefore, at present no satisfactory method for the removal of solid particulate waste from existing cage designs without significant increase in operational costs.

A more simplistic approach involving the use of sub cage collection vessels has been examined by many researchers. Large funnel shaped impermeable collectors have been placed below freshwater cages and wastes removed periodically by pump. Using such a system, Tucholski and Wojno (1980) achieved solid waste recovery of 45% but only 15 - 20% of total N and total P was removed. Hartman et al. (1982) were less successful using a similar device, removing only 9 - 40% of solid wastes produced. Kennedy (1987) reported waste removal efficiencies of *c.* 19%, based on total feed fed. Furthermore, Ackefors and Enell (1994) reported a similar design to that of Kennedy (1987) developed by a Swedish company (Viking Fish AB) for the collection and reuse of feed pellets. Results indicated that 70% of waste P was recovered while the saving in lost feed resulted in a 25 – 30% reduction in feed costs. However, as with the collection system used by Kennedy (1987), it was laborious to install and remove and very prone to fouling. The

reuse of feed pellets proved impossible due to contamination with faecal matter and subsequent unpalatablity (Beveridge, 1996; SNIFFER, 1998).

A Norwegian company, Lift Up Akva, developed a meshed net collector, namely the "Lift-Up" system, and overcame the problems encountered by using solid walled collection systems, as water currents were not affected so badly and allowed for easier handling of the device. The collection system was tested in an empty cage and found to have almost 100% removal efficiency for feed pellets sized 6 mm or larger and 70% efficiency for pellets sized 4 mm (Ervik et al., 1994; SNIFFER, 1998). The system, however, was designed for the collection of waste feed pellets and dead fish and has not been evaluated for its ability to remove faecal waste material.

Behmer et al. (1993) assessed mesh sub cage collection devices for the removal of solid wastes and P from the freshwater cage production of rainbow trout. Pilot scale cages (1.45 m^3) with cone bottom collectors (0.25 m^3) were shown to retrieve *c.* 3% waste solids (as percent of feed fed) in water currents exceeding 0.5 m s^{-1}. The collection efficiency was enhanced to 8% when cages were placed in an area of negligible water currents. This was further enhanced to 16% when pumping of wastes from the collector was increased from 72 h to 24 h. A summary of results from previous studies of waste collection at aquaculture cages is presented below (Table 2.1).

Table 2.1. Removal efficiencies of waste collection systems (expressed as percent of feed added to cage system).

Solid waste (%)	Nitrogen (%)	Phosphorus (%)	Species	Source
45	15 - 20	15 - 20	*S. gairdneri*	Tucholski and Wojno, 1980
9 - 40	--	--	*O. mykiss*	Hartman et al., 1982
19	--	--	*S. salar*	Kennedy et al., 1987
16	--	35	*O. mykiss*	Behmer et al., 1993
--	--	70	*O. mykiss*	Ackefors and Enell, 1994
90*	--	--	*	Ervik et al., 1994

* Note: Lift Up system test in empty fish cage and found to remove almost 90% of feed pellets typically used in Norwegian aquaculture.

2.4. WASTE TREATMENT AND DISPOSAL

Wastewater from the intensive aquaculture process, even with waste minimisation strategies in place, is contaminated with various by-products which must be removed or reduced in concentration before release to the environment.

Treatment methods developed for aquaculture in the past have focussed mainly on the removal of the solid waste fraction due to its biological oxygen demand (BOD), and association with nutrients such as N and P (Alanärä et al., 1994). Mechanical separators, especially screens, are common methods employed to sieve out SS. Solids separation by means of sedimentation is also employed in aquaculture units. Settlement treatment systems work on the principle that solid particles with a density greater than that of water will settle out of suspension from the main water flow (Weston et al., 1996; Cripps and Bergheim, 2000). The efficiencies of both screen and settling treatment methods are

dependent on the concentration and particle size of solids in the waste stream. Separation of particulate matter is greatest with high concentrations and large solid particle size (Kelly et al., 1997; Bergheim et al., 1998). Other methods used include dissolved air flotation (DAF) in combination with chemical flocculation (Jokela and Heinanen, 2000).

The resulting sludge from these treatment methods must then be disposed. There are several proposed possible disposal routes for aquaculture wastes namely land spreading as a fertiliser, land filling, composting, and chemical and biological stabilisation (Chen et al., 1997; Summerfelt et al., 1999; Cripps and Bergheim, 2000). Ideally, the best means of disposal should enable the use of aquaculture effluents as other valuable products, thus increasing the sustainability of the aquaculture process.

Disposal to landfill only serves to move the waste in space and time (leaching of waste in future) with no beneficial return. One of the most common reuse routes for solid aquaculture effluents is through land spreading (Chen et al., 1997). Landspreading has potential disadvantages in that rainfall can result in run off of nutrients to adjacent waterways with an adverse impact on the environment. Odour problems have also been reported and there is also the financial burden and difficulty in transporting the sludge due to it's high water content of over 90% (Summerfelt et al., 1999). Concerns with land application also include the potential risk to public health from pathogenic organisms that may enter the food chain (Burge and Marsh, 1978; Ahmed and Sorensen, 1995; Ponugoti et al., 1997). A further risk in using aquaculture wastes as a land fertiliser is the potential spreading of the pathogen to wild fish stocks due to land run off.

Ideally, waste effluents should be disposed of in such a way that they have a minimum adverse but maximum beneficial impact on the environment. Stabilisation of the waste is thus of prime importance in reducing the pollution potential and putrescence of wastes.

This is usually effected biologically, but chemical methods have been used. Biodegradation, carried out in the presence (aerobic) or absence (anaerobic) of oxygen, reduces the content of organic matter and removes/reduces offensive odours. Aerobic decomposition, for example composting, can reduce waste volume by 50 - 85% and properly composted sludge is essentially pasteurised, odourless and can be marketed for use as a soil conditioner (Chen et al., 1997). However, the composting process is labour intensive and any return on investment will rely heavily on efficient marketing of the product.

Anaerobic digestion is an alternative means of disposal in which stabilisation of waste occurs through fermentation of organic material to a gas consisting mostly of methane (CH_4), which may be readily utilised as a fuel. Furthermore, it has the potential for pathogen inactivation. Anaerobic treatment of waste has the advantage over aerobic decomposition in that a variable amount of energy may be recovered whereas aerobic treatments are merely net consumers of energy.

2.5. WHAT IS ANAEROBIC DIGESTION?

Anaerobic digestion is one of the oldest processes used for the stabilisation of organic material. It may be defined as the use of microbial organisms in the absence of oxygen, for the stabilisation of organic materials by conversion to CH_4 and inorganic products including CO_2:

$$Organic\ \ Matter + H_2O \rightarrow CH_4 + CO_2 + New\ \ biomass + NH_3 + H_2S + Heat$$

This process occurs in different natural environments such as rivers, lakes and ocean sediments, soils and the gastrointestinal tract of animals (Novaes, 1986). As with all biological treatment systems, anaerobic digestion utilises this microbiological process at an intensive level optimising the environmental requirements of biological populations present. The anaerobic fermentative process has a number of advantages namely the reduction in the mass of solid organic material with a concurrent reduction in odours and pathogen concentration. The most beneficial aspect of the process however is its unique ability to produce a net energy gain in the form of CH_4 gas thus optimising cost effectiveness.

2.6. MECHANISM OF ANAEROBIC DIGESTION

In the anaerobic digestion process the biological conversion of organic material to CO_2 and CH_4 occurs involving many genera of bacteria in a complex microbiological process. The anaerobic fermentative process may be described as a three step process (Parkin and Owen, 1986; Metcalf and Eddy, 1991) reportedly involving five groups of bacteria (Figure 2.1). However, this is merely for ease of process description whilst in reality all

bacterial groups are working simultaneously and synergistically with the efficiency of the metabolism of each group dependent on the others (Parkin and Owen, 1986; Massé and Droste, 2000).

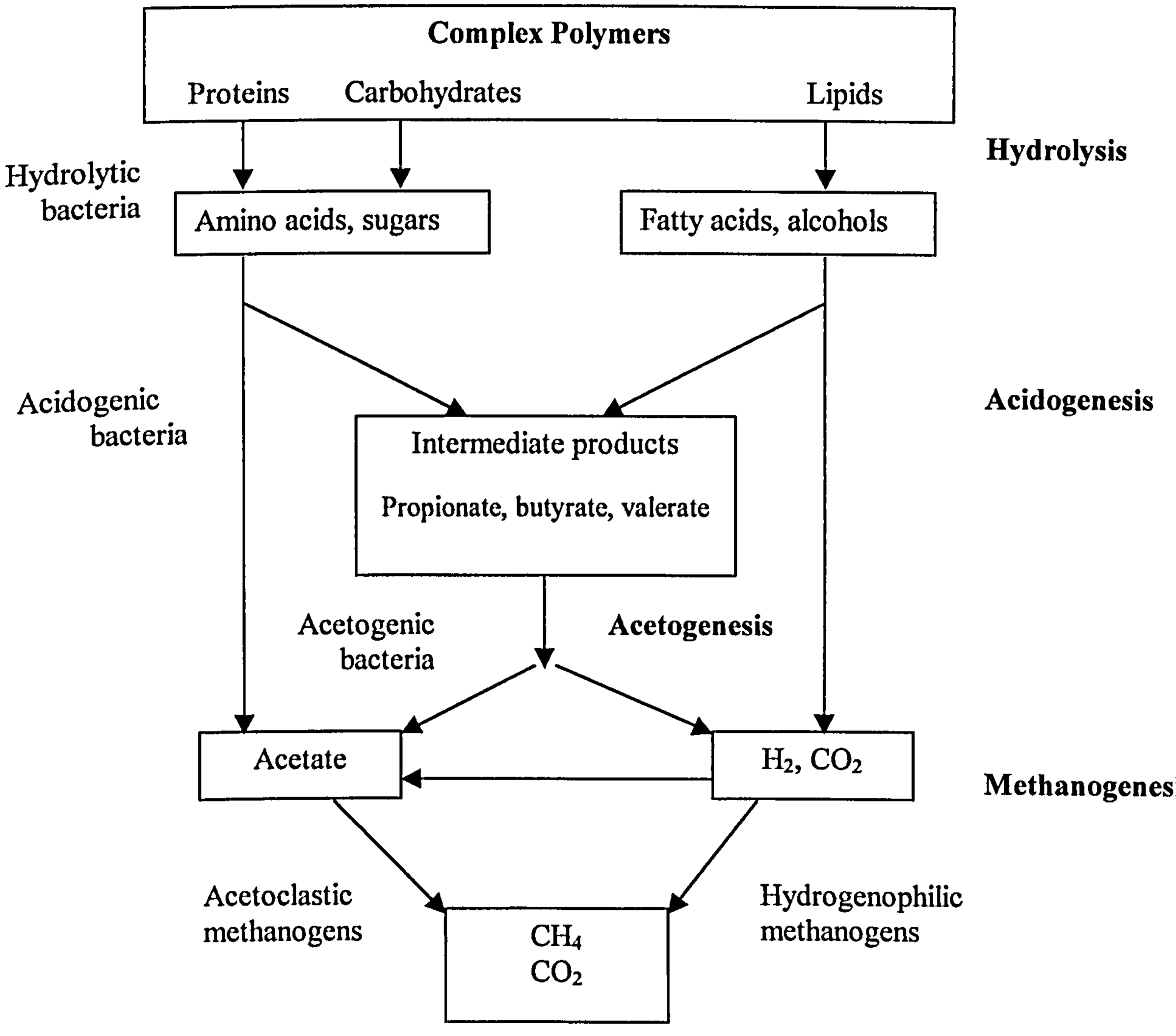


Figure 2.1. Stages in CH_4 production from organic waste during anaerobic digestion.

Initially, most of the constituents of wastewater are not directly available for assimilation by bacteria. Complex organics such as proteins, carbohydrates, lipids and fats are hydrolysed by extracellular enzymes produced by fermentative bacteria to simpler soluble products of a size small enough to allow their passage across the cell membrane of the micro-organsims. These simple compounds of amino acids, sugars, fatty acids and alcohols are then fermented to short chain fatty acids (such as acetic, propionic, and butyric acid), alcohols, ammonia, hydrogen (H_2) and CO_2 through a process called acidogenesis. Organic acids produced during this phase (not already in acetate form) are converted to acetate and H_2 by acetogenic bacteria (acetogenesis). In addition, part of the available H_2 and CO_2 is converted to acetate during acetogenesis. The groups of bacteria involved in these stages may be collectively known as non-methanogens or acid formers and consist of facultative and obligate anaerobes (McCarty, 1964; Novaes, 1986; Kiely, 1997).

During the final stage, strictly anaerobic methanogenic bacteria convert the products of hydrolysis and acidogenesis to CH_4 and CO_2. CH_4 bacteria can only use a few substrates as an energy source. Presently, it is known that only CO_2, H_2, formic acid, acetate, methanol, methylamines and carbon monoxide can act as energy sources during the methanogenesis stage (Metcalf and Eddy, 1991). The two principal pathways for CH_4 formation are from the conversion of acetate by acetoclastic methanogens and from H_2 by hydrogenophilic methanogens, with an estimated 72% of the CH_4 formed coming from the acetate cleavage (McCarty, 1964; Parkin and Owen, 1986). CH_4 is essentially insoluble in water and readily escapes from the sludge as gas. CO_2 is also produced and either escapes as gas or is converted to bicarbonate alkalinity (Novaes, 1986; Parkin and Owen, 1986; Metcalf and Eddy, 1991; Kiely, 1997).

In the anaerobic degradation process, the acid formers and the methanogens have a synergetic relationship in which the methanogens convert fermentation end products resulting from acidogenesis to CH_4 and CO_2. In return, methanogenic bacteria utilise H_2 produced by the acid forming bacteria, which if allowed to increase in the system ($>10^{-4}$ atm) will result in the inhibition of both acidogensis and CH_4 production (Novaes, 1986; Parkin and Owen, 1986). Therefore to maintain an anaerobic treatment system, non-methanogenic and methanogenic bacteria must remain in dynamic equilibrium. Methanogens are sensitive to low pH levels and if it falls below *c.* 6.3 (due to the accumulation of organic acids as a result of acidogenesis) or increases above 7.8 (due to a shift in ammonium (NH_4^+) to the toxic, unionised NH_3 form), CH_4 production rapidly decreases (Lay et al., 1997). Furthermore, the residence time of the biomass within the system must be sufficient to maintain a viable quantity and concentration of bacteria to allow conversion of organic matter to CH_4 and CO_2 and prevent bacterial washout from the system. This is particularly important for methanogens as they have a slower growth rate than acid forming bacteria (Dague et al., 1970). Thus to ensure a stable anaerobic system, the optimal environmental conditions (Table 2.2) within the anaerobic reactor such as correct pH of the aqueous environment in the reactor, absence of dissolved oxygen (DO), heavy metal and sulphides. Importantly, there must be sufficient bicarbonate alkalinity present to neutralise acids. Bicarbonate is formed when CO_2, which is soluble in water, reacts with hydroxide ions (OH^-) to form bicarbonate ions (HCO_3^-). A minimum level of 1,000 mg l^{-1} should exist with volatile fatty acid concentrations less than 250 mg l^{-1}. There must also be sufficient nutrients such as P and N present for proper growth of bacteria. Souza (1986) recommended a C:N:P ratio of 150:5:1 for anaerobic system. Furthermore, temperature is an important environmental parameter with possible operating ranges of 0 - 20°C (psychrophilic), 30 - 38°C (mesophilic) and 50 - 60°C (thermophilic) (Metcalf and Eddy, 1991; Kiely, 1997).

Table 2.2. General Operating and loading conditions for optimum anaerobic digestion of solid wastes (Hammer and Hammer, 1996).

Temperature°C	
Range	0 - 60
Optimum	34 - 37, 54 - 60
pH	
Optimum	7.0 - 7.1
General limits	6.7 - 7.4
Alkalinity concentration, mg l^{-1}	
Normal operation	2,000 - 3,500
Volatile acids concentration, mg l^{-1}	
General operating range	200 - 800
Volatile solids loading, m^3 kg^{-1} d^{-1}	
Conventional single stage	0.5 - 0.6
High rate	1.6 - 6.4
Volatile solids reduction, %	
Conventional single stage	50 - 70
High rate	50
Solids retention time, d	
Conventional	30 - 90
High rate	10 - 20
Gas production, m^3 kg^{-1} d^{-1}	
Per kg volatile suspended solids (VSS) added	0.5 - 0.7
Gas composition, %	
Methane	65 - 69
Carbon dioxide	31 - 35
Hydrogen sulphide	<1

In addition, it most also be remembered that there exists fundamental differences between the digestion of solid and liquid wastes. The initial conversion of complex polymers (*e.g.* proteins) contained in liquid wastes to simpler soluble products (*e.g.* amino acids) will be a relatively fast reaction in comparison with the initial hydrolysis of solid wastes. This phase is often the rate-limiting step in the digestion of solids wastes due to the necessity to break down solid particles to a size small enough to ensure adsorption across the cell membrane. Conversely, the conversion of organic acids to methane is often the rate-limiting step for liquid wastes. Due to the initial rapid fermentation rate, a build up of

organic acids may occur, thus inhibiting the digestion process if methanogenesis does not occur in equilibrium with the fermentation stage.

2.7. REACTOR DESIGN

In order to maintain a stable and efficient operating digestion system, it is important to consider the microbiology of the process. A poorly designed reactor may lead to bacteria inhibition, as described earlier (*Section 2.6*), resulting in a "stuck" digester. For optimal reactor design, biological solids retention time (SRT) is a key factor. SRT is defined as the mass of solids contained in the reactor and the rate of solids removal per day:

$$SRT = \frac{mass\ of\ solids\ in\ reactor, kg}{rate\ of\ solids\ removed, kg\ day^{-1}} \quad \text{.....(Equation 2.1)}$$

The typical minimum SRT for wastewater sludges is 10 days, due to the corresponding growth of methanogenic bacteria over this time period (Owen, 1964; Dague et al., 1970; Kiely, 1997). The longer the SRT the greater the production of CH_4 and hence process efficiency (Parkin and Owen, 1986).

Fundamental to the design of an anaerobic reactor process is determination of the volume required. This may be achieved through a number of methods such as the loading rate of organic material to the system. The most common loading factors used are the volatile solids (VS) and chemical oxygen demand (COD) loading rate:

$$VS\ loading\ rate = \frac{volatile\ solids\ added\ daily, kg\ VS\ day^{-1}}{working\ volume\ of\ the\ digester, m^3} \quad \text{.....(Equation 2.2)}$$

In applying the loading factor, the hydraulic retention time should also be considered due to its relationship to bacterial growth and bacterial washout from the reactor:

$$HRT, \theta = \frac{working\ \ volume, L}{rate\ \ of\ \ sludge\ \ removed, L\ day^{-1}} \quad \text{.....(Equation 2.3)}$$

At a given digester waste feed concentration, lower hydraulic retention times result in higher organic loading rates.

Furthermore, critical to the design of a reactor is an understanding of the kinetics of digestion such as the rate of bacterial growth and decay, and substrate utilisation or waste stabilisation. Establishing appropriate kinetic constants should lead to the optimum digester design (Parkin and Owen, 1986; Linke, 1997). For biological systems such as anaerobic digestion, a mixed culture of microorganisms are present. The rate of growth of bacterial cells, or biomass, of such systems may be defined as:

$$r_g = \mu X \quad \text{.....(Equation 2.4)}$$

where: r_g = rate of bacterial growth (mass unit volume^{-1} time)

μ = specific growth rate (time^{-1})

X = concentration of microorganisms (mass unit volume^{-1})

Substituting $r_g = \frac{dX}{dt}$ gives the first-order rate expression for the rate of biomass increase as:

$$\frac{dX}{dt} = \mu X \quad \text{.....(Equation 2.5)}$$

There are essential requirements for the growth of microorganisms such as the availability of substrate or nutrients. If an essential nutrient or substrate is limiting, then in turn microbial growth is limited. In 1949 Monod showed experimentally that growth rate was a function of organism concentration and limiting substrate or nutrient concentration and proposed the following expression:

$$\mu = \mu_m \frac{S}{Ks + S} \quad \text{.....(Equation 2.6)}$$

where: S = concentration of limiting substrate (mass volume^{-1})

μ_m = maximum growth rate (d^{-1})

K_s = half saturation constant or the concentration of S

when: $\mu = \frac{\mu_m}{2}$ (mass volume^{-1})

However, the Monod expression only assumes the growth of microorganisms, and does not represent the simultaneous decay of biomass. To take account of this, an endogenous decay (rate = k_d, day^{-1}) is used and therefore the growth rate of biomass may be represented as:

$$\frac{dX}{dt} = (\frac{\mu_m S}{Ks + S})X - k_d X \quad \text{.....(Equation 2.7)}$$

If all the substrate could be converted to biomass then substrate utilisation would equal rate of bacterial growth:

$$-\frac{dS}{dt} = \frac{dX}{dt} \quad \text{.....(Equation 2.8)}$$

However, due to inefficiencies in the conversion process, not all of the substrate can be transformed to biomass so a yield coefficient (Y<1) is incorporated into the equation such

that rate of substrate utilisation is in excess of rate of biomass production giving the expression for substrate utilisation as:

$$-\frac{dS}{dt} = \frac{1}{Y}\left(\frac{\mu_m SX}{Ks + S}\right) \qquad \text{.....(Equation 2.9)}$$

where: $Y = \frac{dX}{dS}$ = fraction of substrate converted to biomass (mass unit volume^{-1} of biomass mass^{-1} unit volume^{-1} of substrate).

For a completely mixed reactor with no solids recycle (SRT = HRT) and assuming no influent biomass (Figure 2.2), a mass balance for net biomass growth and substrate utilisation (waste stabilisation) can be expressed as follows:

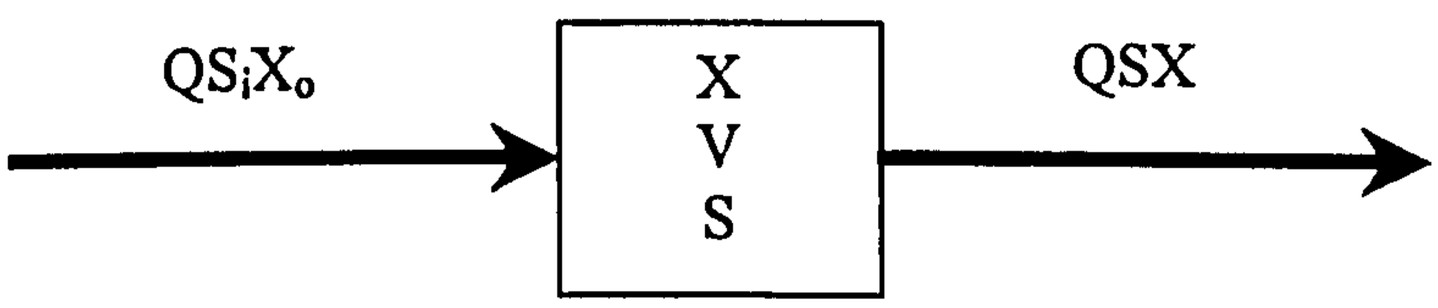


Figure 2.2. Schematic of completely mixed reactor without solids recycle.

Where: Q = volumetric flowrate (m^3 day^{-1});

S = substrate concentration (kg m^{-3});

S_i = initial substrate concentration (kg m^{-3});

X = mass concentration of microorganisms (g kg^{-1});

X_o = Initial mass concentration of microorganisms (g kg^{-1});

V = volume of reactor (m^{-3})

Mass balance:

$$QX_0 + \frac{dX}{dt}V = QX \qquad \text{.....(Equation } 2.10\text{)}$$

Substituting for $\frac{dX}{dt}$ and assuming no influent biomass:

$$\frac{\mu_m S}{Ks + S} - k_d = \frac{QX}{VX} = \frac{1}{\theta} \qquad \text{.....(Equation } 2.11\text{)}$$

As SRT is equal to HRT (θ) for a complete mixed system with no solids recycle, it is shown that the growth rate of biomass is related to the SRT of the system.

Similarly, substrate utilisation may be represented as:

$$\frac{dS}{dt}V = (Si - S)Q \qquad \text{.....(Equation } 2.12\text{)}$$

Substituting for $\frac{dS}{dt}$ gives the expression:

$$\frac{\mu_m S}{Ks + S} = \frac{QY}{VX}(S_i - S) \qquad \text{.....(Equation } 2.13\text{)}$$

This may be rearranged incorporating SRT and to yield biomass concentration:

$$\frac{QX}{VX} + k_d = \frac{QY}{VX}(Si - S) \qquad \text{.....(Equation } 2.14\text{)}$$

Noting that $k = \frac{\mu_m}{Y}$ and rearranging:

$$X = \frac{Y(S_i - S)}{1 + k_d\theta} = \frac{\mu_m(S_i - S)}{k(1 + k_d\theta)} \quad \text{.....(Equation 2.15)}$$

Similarly, incorporating SRT and solving for S, substrate effluent concentration may be expressed as:

$$\frac{\mu_m S}{Ks + S} - k_d = \frac{1}{\theta} \quad \text{.....(Equation 2.16)}$$

Therefore:

$$S = \frac{Ks(1 + \theta k_d)}{\theta(Yk - k_d) - 1} \quad \text{.....(Equation 2.17)}$$

If the kinetic coefficients, such as Y, k, and k_d are known for the above expressions, they can be used to predict the effluent microorganism and substrate concentrations and hence the optimum SRT for the anaerobic digestion process. Substrate concentration, S, is generally monitored as COD or VS. Bacterial concentration, X, may be estimated by measuring DNA, protein and ATP content, but volatile suspended solids (VSS) is most commonly used due to its simplicity (Metcalf and Eddy, 1991). It should be noted however that the above expressions are based on soluble waste and do not consider the effect of influent suspended solids. Furthermore, bacterial VSS cannot be separated readily from other types of VSS, representing a significant analytical problem in the determination of reaction rates for substrate utilisation (Chen and Hashimoto, 1980; Parkin and Owen, 1986).

Alternatively, the rate of waste stabilisation, and hence the retention time required for optimum process efficiency may be monitored by examining the rate of CH_4 production. Analysis of CH_4 production may be used to predict the biodegradable substrate concentration as function of retention time and organic loading (Chynoweth et al., 1982). The quantity of gas produced is directly related to the destruction of organic matter (Parkin and Owen, 1986). The anaerobic degradation of waste is generally assumed to follow a first order rate of decay (Owens and Chynoweth, 1993; Merkel et al., 1996; Sanchez et al., 1996; Valentini et al., 1997; Lin et al., 1999; Rodriguez-Andara et al., 1999) and thus the production of CH_4 is assumed to be first order also. The expression for CH_4 production may be derived as follows:

Rate of substrate degradation:

$$\frac{dS}{dt} = -kS \quad \text{.....(Equation } 2.18\text{)}$$

Chen and Hashimoto (1978) proposed the following CH_4 to substrate equation of proportion:

$$\frac{CH_{4\max} - CH_4}{CH_4} = \frac{S}{So} \quad \text{.....(Equation } 2.19\text{)}$$

Dividing equation *2.18* by *So*, and substituting *So* with equation *2.18* and integrating gives the expression:

$$\ln[(CH_{4\max} - CH_4)/CH_{4\max}] = -kt \quad \text{.....(Equation } 2.20\text{)}$$

Which may be expressed alternatively as:

$$CH_4 = CH_{4max}\,(1 - e^{-kt}) \quad \text{.....(Equation } 2.21\text{)}$$

where:

CH_{4max} = ultimate CH_4 yield

CH_4 = cumulative CH_4 yield at time t

k = first order rate constant

Based on the CH_4 production per gram of substrate destroyed (as COD or VS), the quantity of substrate at time *t* can be calculated using the CH_4 production data (Lin et al., 1999). Once CH_{4max} is known, the CH_4 production expression may be linearised, plotting $\ln \frac{CH_4}{CH_{4\max}}$ versus *t*, and the coefficient *k*, may be calculated from the slope of the resulting linear equation (Rodriguez-Andara et al., 1999).

The calculation of the CH_4 yield may be achieved through batch digestion using the biochemical methane potential (BMP) assay. The assay was developed by Owen et al. (1979) to estimate the ultimate conversion and associated CH_4 yield of organic substrates. The method has been applied successfully to determine the ultimate CH_4 production from a variety of feedstocks (Owen et al., 1979; Deren et al., 1991; Chynoweth, et al., 1993; Owen and Chynoweth, 1993; Ergüder et al., 2000) and the determination of kinetic coefficients for the digestion process (Lin et al., 1999).

Other factors for consideration in the design process include operation temperature, gas production and rate of mixing. Increasing the temperature of the digestion process will result in a greater degradation and biogas production (van Velson and Lettinga, 1979; Van Lier et al., 1990; 1992; 1997; Nozhevnikova et al., 1999; Massé and Massé, 2001) and hence allow for greater loading rates. Biochemical reaction rates, as predicted by the van't Hoff equation, increase as temperature increases, approximately doubling for every 10°C rise up to a point (beyond which inactivation due to excessive temperatures may occur), therefore an increase in digester operating temperature will result in an increase in digestion performance (Maly and Fadrus, 1971; Massé and Massé, 2001). Optimum

temperatures for digestion are in the mesophilic and thermophilic range. Thermophilic operation has the advantage of requiring a smaller digester size at a given loading in comparison with mesophilic. Also, higher temperatures result in substantial pathogen inactivation (Cooney, 1975; Lee et al., 1989; Aitken and Mullennix, 1992; Watanabe et al., 1997), but increased costs will be incurred for the supply of additional heat.

Similarly, mixing has an important impact on digester performance allowing greater utilisation of reactor volume and preventing stratification and temperature gradients. Most importantly, it maintains intimate contact between bacterial populations and the substrate (Parkin and Owen, 1986).

2.8. PROCESS DESCRIPTION

Anaerobic digestion is performed in an air tight container, generally cylindrical in shape, with sludge being introduced continuously or periodically and retained in the reactor for varying periods of time (Metcalf and Eddy, 1991). There are two basic reactor process designs in anaerobic waste treatment, namely conventional or standard rate and high rate digestion. In the conventional process, the digester contents are usually unmixed and unheated and have long retention times varying from 30 - 60 days (Metcalf and Eddy, 1991; Hammer and Hammer, 1996). In contrast, high rate digesters are characterised by continuous mixing, except at times of sludge removal, by means of gas recirculation or mechanical mixers and the sludge is heated to enhance the digestion rate (Figure 2.3). The solids loading to the reactor is higher and the retention time is typically one half that of low rate digestion (Davis and Cornwell, 1995; Kiely, 1997). There are many variations of this basic type process design and the different reactor configurations have been classified

by Kiely (1997) based on the HRT and SRT. Systems in which the SRT is equal to the HRT include:

1. Batch digester;
2. Plug flow digester;
3. Continuously stirred tank reactor (CSTR);
4. Anaerobic contact reactor.

Reactor configurations (Figure 2.3) in which the SRT is greater than the HRT include:

1. Upflow-downflow anaerobic filter;
2. Downflow stationary fixed film reactor;
3. Fluidised bed reactor;
4. Upflow anaerobic sludge blanket reactor (UASB);
5. Hybrid anaerobic sludge reactor.

A further means of classifying anaerobic reactors is the characteristics of the waste stream for treatment, which will determine the reactor configuration employed. Readily degradable and soluble wastes of about 2 - 6 % solids content, for example industrial wastewaters, are generally treated using contact or filter processes such the UASB, the fluidised bed and the anaerobic filter reactors (Kiely, 1997). Very low retention times from between a few hours to only a few days may be achieved with high organic loading rates. High solids waste effluents with typically 20 - 30% solids content such as municipal and agricultural wastes require long retention times typically 10 - 30 days (Metcalf and Eddy, 1991; Hammer and Hammer, 1996; Kiely, 1997; Oleszkiewicz and Poggi-Varaldo, 1997). Complete mix reactors with intermittent or continuous feeding are often used for high solids digestion (Kayhanian and Rich, 1995).

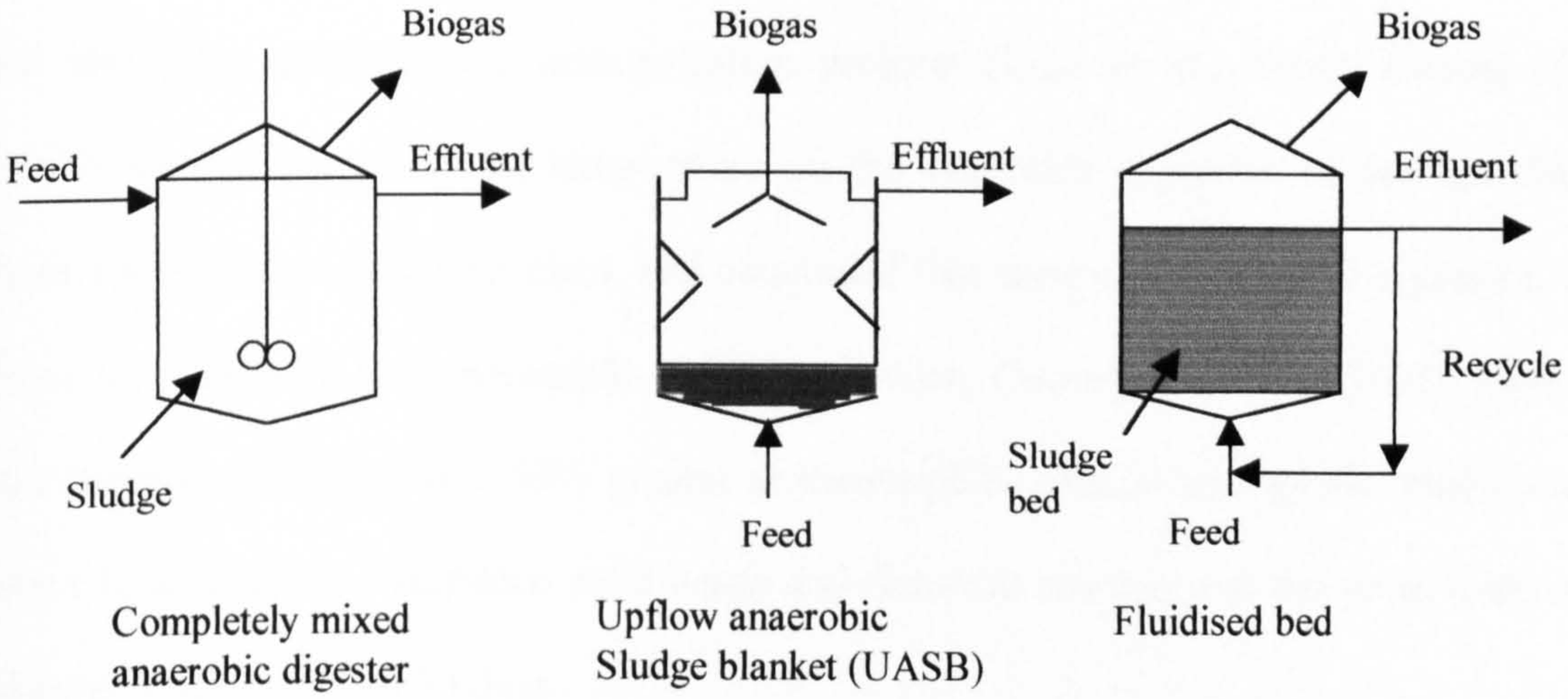


Figure 2.3. Schematic of typical reactor configurations used in the anaerobic digestion of wastewater.

2.9. APPLICATION OF ANAEROBIC DIGESTION

Due to the large environmental impacts of landfills, many of them are due to close in Europe with the preparation of legislation to restrict its practice (Mata-Alvarez et al., 2000). In the UK, the disposal fee to landfill is also set to increase discouraging its use and emphasising the need to recycle and recover waste components. Anaerobic digestion is one such application that allows waste recovery and literature is heavily documented with a diverse range of its applications.

Anaerobic digestion has for years been used to stabilise the organic fraction of municipal solid waste (OFMSW), reduction in waste volume and potential for energy production being the main attractions (Lepistö and Rintala, 1995). Presently, it is estimated that there are over 36,000 anaerobic digesters in operation in Europe, treating around 40 - 50% of

municipal sewage sludge generated (Mata-Alvarez et al., 2000). There is a considerable quantity of published literature on this subject, covering not only laboratory-scale work but also pilot and full-scale demonstration projects (Lusk et al., 1996). Cecchi et al. (1992) studied the effects of temperature on the anaerobic digestion of sewage sludge from a wastewater treatment plant, and concluded that thermophilic (55°C) operation was more cost effective than mesophilic (35°C) operation. Cooney and Wise (1975) reported that biogas production was 50% greater at thermophilic than at mesophilic temperatures when of a mixture of shredded solid waste and domestic sewage was fed to an anaerobic digester with a HRT of 30 days.

The use of anaerobic digestion to treat certain industrial and agricultural wastes is also well documented. Oleszkiewicz and Poggi-Varaldo (1997) achieved 44% - 53% VS destruction when a mixture of solid municipal waste and industrial waste containing 30% total solids (TS) was digested under thermophilic conditions. The biogas produced contained 60% CH_4 by volume. The anaerobic digestion of animal wastes has also been examined (Table 2.3). Safley and Westerman (1990) examined the low temperature (14 - 23°C) digestion of animal manure and observed acceptable CH_4 yields for loading rates between 0.15 and 0.57 kg VS m^{-3} reactor volume day^{-1}. Similarly, Massé et al. (1995) obtained 0.48 m^3 CH_4 kg^{-1} VS of swine manure slurry fed to a psychrophilic anaerobic digester (20°C), overall COD removal being 41 - 83%.

Table 2.3. Summary of literature relating to the anaerobic digestion of animal wastes.

Waste stream	Temperature (°C)	Reactor scale/type	Reference
Cattle manure	Ambient	Full/ CSTR	Sarapatka, 1994
	40 & 60	Bench/ CSTR	Mackie and Bryant, 1995
	25	Bench/ UASB	Peck et al., 1987
	20	Bench/ SBR	Massé et al., 1995
Pig slurry	55	Bench/ CSTR	Hill et al., 1986
	55	Bench/ CSTR	Hansen et al., 1999
Poultry	35	Bench/ CSTR	Webb and Hawkes, 1985
	35	Batch/ bioassay	Magbauna et al., 2001
	55	Full/ CSTR	Collins et al., 2000

Furthermore, a comprehensive report on the biological conversion of both terrestrial and aquatic biomass to CH_4 demonstrated the increasing importance of anaerobic digestion in energy recovery (Gunaseelan, 1997).

Relatively little work has been published on the anaerobic digestion of aquaculture effluents. Van Rijn et al. (1995) examined the degradation of fish feed pellets sealed inside nylon mesh bags of pore size 40 μm and then incubated in an anaerobic digestion basin at 25°C. They found that 85% of the carbohydrate, 92% of the protein and 66% of the lipid had been rendered soluble after 20 days, although the results suggested that degradation of the recalcitrant material present could take considerably longer.

Lanari and Franci (1998) studied the possibility of using solid waste from fish farm effluents to generate biogas with the partial recirculation of water. Waste from fish tanks was eluted to settling columns. Supernatant water was treated using an aerobic biofilter and sedimented waste was transferred to a psychrophilic anaerobic digester. Treated effluent from the digester was then passed through a zeolite exchange column in order to

reduce total ammonia nitrogen content before discharge. Results from the study were excellent. At hydraulic retention times between 22 and 38 days, the digester reduced TS, VS and SS by 91 - 93%, 94 - 97% and 96% - 99% respectively. The CH_4 content of the biogas was greater than 80% and as expected biogas production increased as the feeding rate increased. Solids reduction was not affected by an increase in loading rate.

Kugelman and Van Gorger (1991) examined the mesophilic anaerobic digestion of aquaculture waste material and found the process was inhibited due to unionised ammonia. Dilution of the waste 1:1 with water overcame this problem and CH_4 production was *c.* 72% of the theoretical yield at a retention time of 30 days. However, the possible enhancement of the process and optimum design was not evaluated.

2.10. ENHANCEMENT OF ANAEROBIC DIGESTION PROCESS

2.10.1. Nutrient Supplementation of Digester Feed

As discussed earlier (*Section 2.6 and 2.7*), critical to the optimum stable digestion process is proper design and optimum environmental conditions for the growth of microorganisms. The growth and maintenance of anaerobic bacteria is critical to the performance of the anaerobic digestion process. To achieve efficient anaerobic biodegradation, a large stable viable bacterial population must be maintained. One of the key factors in achieving this is substantial bacterial contact with substrate nutrients. For optimal metabolism, bacteria require effecient concentrations and ratios of certain nutrients. These nutritional requirements may be categorised as either macro or micronutrients and their functions in anaerobic digestion are summarised in Table 2.4.

Table 2.4. Functions of nutrients in anaerobic digestion (Kayhanian and Rich, 1995).

Nutrient	Function
Carbon, C	Basic building block of bacterial cell material and primary source of energy.
Nitrogen, N	Primary nutrient required for protein synthesis
Phosphorus, P	Nucleic acid synthesis
Potassium, K	Increases cell wall permeability
Sulphur, S	Sulphide required in numerous enzymes
Cobalt, Co	Required in corrinoids and specific enzymes such as carbon monoxide dehydrongenase (CODH)
Iron, Fe	Helps activate numerous enzymes, formation of sulphide precipitates, excretion of extracellular polymers
Molybdenum, Mo	Present in enzyme formate dehydrogensae (FDH) but may inhibit sulphate reducing bacteria
Nickel, Ni	Required by CODH, essential for sulphate reducing bacteria, aids CO_2 and H_2 conversion
Selenium, Se	Present in enzymes such FDH, Se enzymes aid in the metabolism of fatty acids
Zinc, Zn	Present in FDH and hydrogenase.

Speece and McCarty (1964) calculated the N and P requirements using an average chemical formulation for biological cells. They estimated the N requirement to be 11% of bacterial cell volatile solids weight and P to be approximately 20% of the N requirement. Other elements required in low concentrations (μg) include calcium, magnesium, cobalt, nickel, and iron. It should be noted that all of these elements in Table 2.4 would exhibit inhibitory effects at high concentrations.

Literature is well documented with examples of the need for nutrient supplementation. Dinsdale et al. (1996) stimulated the mesophilic anaerobic digestion of coffee ground wastes by the addition of calcium hydroxide, N, P and a micro nutrient solution of trace metals. They found that they could not achieve long term stable digestion without addition of nutrients. This was attributed to the high lipid levels in the coffee waste which are solubilised to long chain fatty acids (LCFA) which at high concentrations are toxic to methanogenic bacteria (Angelidaki et al., 1990). The continuous digestion of coffee waste with nutrient supplementation achieved greater process stability along with an increase in biogas production from 0.2 l l^{-1} day^{-1} to 0.34 l l^{-1} day^{-1}. Volatile fatty acid (VFA) concentration was also reduced from >2,000 mg l^{-1} to an average of 100 mg l^{-1}.

Further examples of the effects of nutrient deficiencies were also cited by Jarvis et al. (1997) for the poor biogas production in the anaerobic digestion of silage. Since cobalt was the only trace element to enhance the methanogenesis process in batch assays performed on sludge samples from the silage digestion process, it was supplemented in the feed. CH_4 yield and process stability were improved as a result. CH_4 production was increased from 0.2 l g^{-1} VS to 0.3 l g^{-1} VS. A greater organic loading rate was also achieved increasing from 5.0 g VS day^{-1} to 7.0 g VS day^{-1}.

2.10.2. Pre-treatment of Digester Feed

As described earlier (*Section 2.6*), the anaerobic treatment process can be described as a three step process with two distinct phases, initial solubilisation and fermentation followed by methanogenesis. Essentially no stabilisation of waste occurs during hydrolysis as organic matter is simply converted into a soluble form that can be utilised by bacteria (McCarty, 1964). The solubilisation of solid organic material is widely

regarded as the rate-limiting step in the anaerobic digestion of solid wastes (Pfeffer, 1979; Eastman and Ferguson, 1981; Parkin and Owen, 1986; Wang et al., 1997). The anaerobic digestion process may therefore be improved if the rate-limiting step of hydrolysis can be enhanced. This may be achieved by increasing the solubilisation of cell bound organic matter resulting in an increase in sludge biodegradability. Lawler et al. (1986) stated that the rate of hydrolysis is directly related to the surface area of the sludge particles. It can, therefore, be assumed that any increase in sludge particle surface area will increase the rate of hydrolysis (Quarmby et al., 1999). There have been many methods employed to augment the hydrolysis step such as thermal treatment (Stuckey and McCarty, 1978; Li and Noike, 1992), ozonation (Yasui and Shibata, 1994), and chemical solubilisation (Woodward and Wukasch, 1994; Chiu et al., 1997; Lin et al., 1997).

Ultrasonication has been increasingly used recently as a pre-treatment method for anaerobic digestion. Ultrasonication causes a localised pressure drop in the aqueous phase causing it to fall below the evaporating pressure resulting in the formation of micro bubbles by evaporation. These micro bubbles oscillate in the sound field, grow by rectified diffusion and collapse in a non-linear manner. The combination of bubble oscillation and the resulting vacuum created by the collapse of the bubble leads to strong mechanical forces that can erode solid particles (Tiehm et al., 1997).

The use of ultrasound as a pre-treatment for mesophilic anaerobic digestion has been previously studied by Tiehm et al. (1997). They showed that at a HRT of 22 days the VS content of anaerobically digested sewage sludge, when pre-treated, could be reduced from 50.3% to 45.8% in comparison with sludge which was not exposed to ultrasonication. Furthermore, the effects of sonication were also shown through an increase in effluent soluble COD, demonstrating that ultrasound resulted in the solubilisation of organic material from the solid to the aqueous phase thus making the sludge more biodegradable.

Sonicating sludge can also improve CH_4 production during the anaerobic biodegradation process. Forster et al. (2000) examined the effects of ultrasound pre-treatment on the thermophilic anaerobic digestion of waste activated sludge (WAS) and found a 15% increase in CH_4 yield. These results compare well to those reported by Quarmby et al. (1999) (6% for pilot scale reactors) and Clark and Nujjoo, (2000) (5 - 10%).

Previous studies on the effects of ultrasound on sludge treatment have investigated the intensity of the ultrasonic frequencies (King and Forster, 1990; Quarmby et al., 1999; Forster et al., 2000). It has been shown that there is clear relationship between sonic intensity and the resulting mean particle size (King and Forster, 1990). Forster et al. (2000) have recommended an optimum sonic dose of 1.5 - 3.0 kJ g TS^{-1}, based on residual turbidity and optimum soluble carbohydrate release. However, an increase in ultrasonic intensity from 111 W min to 356 W min has been shown to have a minimal effect (3.5%) on the digestion process in terms of gas production (Quarmby et al., 1999).

2.10.3. Co-digestion of Waste Effluent

Further means of enhancing the solid waste digestion process include co-digestion. This involves the use of a co-substrate which will improve the performance of the digestion process and ultimately the biogas yield due to positive synergisms established in the digestion medium and the supply of missing nutrients by the additional substrate (Mata-Alvarez et al., 2000). Magbanua et al. (2001) reported an increase from 0.027 to 0.200 l g^{-1} VS added for the co-digestion of hog manure with a 20% mixture of poultry waste. Similarly, a recent study demonstrated that fish offal and brewery solids as codigestates with cattle slurry improved the biogas yield from 0.3 m^3 CH_4 kg^{-1} VS_{rem} with cattle slurry alone to *c.* 0.38 and 0.31 m^3 CH_4 kg^{-1} VS_{rem} respectively (Callaghan et al., 1999).

However, the same study also showed the importance of determining the correct type of co-substrate and its digester load. The co-digestion of cattle slurry with 7.5 and 15% TS chicken manure resulted in specific CH_4 yields of 0.16 and 0.13 m^3 CH_4 kg^{-1} VS_{rem} respectively, the high load having a lower yield than cattle slurry alone. This may however have been due to the higher VS load applied for those tests. Another possible reason was the inhibition of methanogenesis by ammonia.

In addition to improving process performance, co-digestion can have economic advantages such as the sharing of equipment and the supplementation of organic waste which at particular sites may not be generated in sufficient quantity to make anaerobic digestion cost effective.

2.11. DISPOSAL OF ANAEROBIC EFFLUENTS

Land application as a fertiliser is a suitable means of disposal for effluents from the anaerobic fermentation of organic material to CH_4 (Hons et al., 1993; Kiely, 1996). Nutrients contained in solid wastes are released in soluble form making them readily available for plant assimilation. However if digestion does not occur efficiently, effluents may have high concentrations of VFA which are phytotoxic, and may not be hygienised if digestion does not occur in the thermophilic range of temperatures (Poggi-Varaldo et al., 1999).

If the treated effluents are to be discharged to surface waters, due to intrinsic process characteristics (Tilche et al., 1996), anaerobic treatment will often require post-treatment in order to meet increasingly stricter discharge standards (Ødegaard, 1988; Penetra et al.,

1999; Mata-Alvarez et al., 2000). In general, secondary treatment processes such as anaerobic digestion are used for the singular purpose of carbonaceous matter stabilisation.

The main objective of anaerobic digestion post-treatment methods is to enhance the removal of residual organic matter and nutrients (Gonçalves et al., 1998). There are a variety of post-treatment methods in use today. Physio-chemical methods such as dissolved air flotation (DAF) in combination with the use of chemical coagulation have been investigated by Penetra et al. (1999). Takashima et al. (1996) also studied the effect of combining membrane separation and alkaline heat post-treatment on the anaerobic digestion of activated sludge. They showed that by using membrane separation alone digester performance was enhanced by 10% in terms of particulate solids degradation. A further increase of 40% in solids decomposition was observed with the use of alkaline heat treatment in combination with membrane separation.

Effluents from the anaerobic treatment process are typically high in ammonia N (NH_4-N) concentrations due to the deamination of organic N in the waste. The presence of ammonia in wastewater entering a water course is undesirable due to its toxicity to fish. The term ammonia refers to two chemical species which are in equilibrium in water (NH_3, un-ionised and NH_4^+, ionised). The balance of this equilibrium is dependent on pH and temperature of the water. As the pH and temperature increase, the percentage of unionised ammonia also increases. The toxicity of ammonia is primarily attributable to the unionised form (NH_3). Acute toxicity can occur at 0.2 mg NH_3 l^{-1} in salmonids and a maximum permissible level of 0.002 mg NH_3 l^{-1} has been recommended (Hagopian and Riley, 1998). Conversion of ammonia to nitrate (NO_3^-) however before discharge will reduce the potential toxic impact of ammonia to aquatic life. NO_3^- is not toxic to fish unless present at high levels (> 100 mg l^{-1}). Further reduction in residual organic material and NH_4-N may be achieved using a simple aerobic biofiltration unit.

2.11.1. Biological Filtration

The treatment of the wastewater via a biofilter is carried out by a complex community of micro-organisms including bacteria, protoza, fungi and algae which form a sponge like "biofilm" together with invertebrate animals. The organisms of the film community live by oxidising organic and inorganic compounds in the wastewater, by feeding upon compounds released by other organisms, on lower forms of life or their dead remains (Metcalf and Eddy, 1991).

The biological filter is termed a fixed film reactors since the biofilm is physically fixed to the surface of an inert medium which provides a high surface area so the biological oxidation and the transfer of oxygen into solution can proceed at optimal rates (Kiely, 1997).

Purification of the wastewater occurs via a number of processes. Carbonaceous solids physically trapped on the medium and those flocculated by secreted extracellular polymers are hydrolysed and absorbed as soluble matter by microorganisms. Solids may also be removed by direct ingestion by protozoa and macro invertebrates. Soluble compounds (organic compounds and ammonia) are assimilated by diffusion and aborption into the biofilm and oxidised by the microorganisms (Metcalf and Eddy, 1991). The mechanism by which ammonia is oxidised to nitrate is known as nitrification.

2.11.1.1. Nitrification

Nitrification is the sequential, two step biological oxidation of ammonia to nitrate (NO_3^-).

$$NH_4^+ + 2O_2 \longrightarrow NO_3^- + 2H^+ + H_2O$$

The process involves two phylogenetically distinct groups of bacteria, ammonia oxidising bacteria or nitrite bacteria (such as *Nitrosomonas*) and nitrite oxidising bacteria (such as *Nitrobacter*). In the first step, ammonium nitrogen is converted to nitrite (NO_2^-) and in the second step, the resulting nitrite is mineralised to nitrate as follows:

Step 1, nitrition:

$$NH_4^+ + \frac{3}{2}O_2 \xrightarrow{Nitrosomonas} NO_2^- + 2H^+ + H_2O + 84\ kcal\,mol^{-1}$$

Step 2, nitration:

$$NO_2^- + \frac{1}{2}O_2 \xrightarrow{Nitrobacter} NO_3^- + 17.8\ kcal\,mol^{-1}$$

Thus, two moles of oxygen are required for each mole of NH_4^+ oxidised. Both reactions are exogenous, the first releasing more than 4 times more energy than the second resulting in a slower growth rate of *Nitrobacter* (Hagopian and Riley, 1998).

Biological post-treatment is by far the most common means of polishing effluents from secondary anaerobic treatment systems. Aerobic biological treatment offers the potential to degrade components such as nutrients, which remain relatively untouched by the anaerobic process (Gonçalves et al., 1998). There are many examples in literature of integrated anaerobic-aerobic systems, which utilise the beneficial attributes of both

individual processes such as low sludge production and nutrient removal. A simple submerged biofilter was shown to improve the digestion of domestic sewage increasing the removal of COD, SS and BOD by 16, 20, and 4% respectively. The biofilter also removed on average 90% of the ammonia nitrogen present in the wastewater (Gonçalves et al., 1998).

Using a more complex design, Tilche et al. (1996) studied the combined use of an anaerobic-anoxic-aerobic system for the treatment of municipal wastewater. This integrated unit, namely ANANOX®, was designed for the optimal removal of organic matter and N. It incorporates an aerobic step for the oxidation of ammonia nitrogen produced during anaerobosis to NO_2^- and NO_3^- and complementary anoxic zone for the conversion of nitrate to atmospheric N_2. Although the system proved successful with 50% lower sludge production and less energy consumption than traditional processes, it required a clarified effluent to be recycled from the activated sludge unit (aerobic zone) to the anoxic zone for dentrification. This may be overcome by the use of a simple biofilter instead of the activated sludge unit.

A comprehensive review of post-treatment of anaerobically treated effluents was made by Ødegaard (1988). Among his conclusions were that a significant amount of particulate organic material (SS) may be removed by improved solid-liquid separation and using membrane filtration techniques such as microfiltration. Furthermore, the aerobic removal of COD/BOD and ammonia can be successful although inhibition of nitrification may occur due to high unionised ammonia concentrations.

2.12. WHY USE ANAEROBIC DIGESTION?

In addition to the stabilisation of organic material with concurrent odour reduction and a decrease in sludge volume, anaerobic digestion offers the potential for energy recovery and the inactivation of pathogenic organisms.

2.12.1. Energy Recovery

The beneficial products of the anaerobic digestion process are primarily a biogas consisting mainly of CH_4 and CO_2 (typically 60% CH_4). The increasing demand for energy worldwide and the resulting depletion of natural resources has increased pressure for the sustainable production of energy from renewable sources. In the UK, government policy has targeted an increase to 10% in the proportion of energy generated from renewable sources by 2010 through strategies such as the Non Fossil Fuel Obligation (NFFO) which give renewable energies a guaranteed premium price (ETSU, 1998). The implementation of the EC Directive 96/61 on Integrated Pollution Prevention and Control (IPPC) will increase the need for the agricultural sector to minimise pollution using the Best Available Technology (BAT). Furthermore, UK government commitments to reduce its greenhouse gas emissions to 12.5% of 1990 emissions between 2008 and 2012 should also increase the interest in alternative waste management methods.

The production of biogas, which has a typical calorific value of 17 – 25 MJ m^{-3}, is therefore of great importance as it can be burned to allow energy recovery either through the generation of electricity or production of heat. It should be remembered, however, that biogas contains trace amounts of other gases such as H_2S (typically <1%). This may result in the corrosion of equipment used in the utilisation of the biogas. Although gas cleansing or scrubbing equipment is expensive, when using the gas for the generation of

electricity, a diesel generator with 10% diesel fuel may be used which lubricates the generator protecting it from corrosion as a result of H_2S (Boyd, 2000; Reynell, pers comm).

The undecomposed solid matter or digestate can be separated into a fibre and liquor. The digestate fibre may be used or sold as a soil conditioner as an alternative to peat. It may require a short period of stabilisation through composting or be applied directly to land depending on its quality. However, the ability to sell the product will depend greatly on the available market.

2.12.2. Pathogen Inactivation

This is of concern if the resulting digested sludge is to be considered for use as a fertiliser as stated in *Section 2.4*. Although the spread of a typical fish bacterial pathogen such as *Yersinia ruckeri* (which causes enteric redmouth disease, see *Section 3.5.1*) to wild fish stocks is unlikely, it has been shown that *Y. ruckeri* is capable of free living in freshwater for up to 4 months, and may also survive in mud for up to 2 months (Austin and Austin, 1993). Therefore, the destruction or inactivation of such bacterial pathogens during the anaerobic digestion process is desirable.

Many researchers have previously investigated the inactivation of pathogens during anaerobic digestion. Ponugoti et al. (1997) showed that under mesophilic operating conditions, a reduction in organisms such as faecal coliforms (FC), Faecal Streptococci (FS) and *Salmonella* of 1 – 3 $\log_{10}$, 1 - 2 $\log_{10}$ and 1 - 2 $\log_{10}$ respectively could be achieved. Similarly, Pedersen (1981) reported a 1 - 2 $\log_{10}$ reduction in the densities of bacteria and viruses during mesophilic anaerobic digestion (Ponugoti et al., 1997). Temperature as expected has a significant influence on the pathogen destruction

capabilities of anaerobic digestion. When increased to thermophilic conditions the efficiency of pathogen reduction is enhanced (Berg and Berman, 1980; Garber 1986; Aitken and Mullennix, 1995; Watanabe et al., 1997). Watanabe et al. (1997) reported no reduction in FC numbers under mesophilic conditions for the digestion of primary sludge. However, under thermophilic temperatures and at a HRT greater than 10 days, a 2 - 4 log_{10} reduction or inactivation of pathogenic bacteria occurred. The mechanism of destruction may be attributed to many environmental parameters but the inhibitory effects of VFA are well documented (Henry et al., 1983; Abdul and Lloyd, 1985; Cherrington et al., 1990; Kunte et al., 2000).

Although all of the treatment systems including anaerobic digestion may be used for both land and water based farms, their ability to reduce the impact of aquaculture effluents on the environment will depend on the efficiency of the sub cage collection system to intercept and collect waste from the cage system.

2.13 The Objectives of this Research

The fundamental aim of this research was to investigate the feasibility enhancing the environmental sustainability of cage aquaculture. This was assessed by examining the use of a simplistic undercage collection device to reduce the dispersion of aquaculture wastes from cages under typical climatic conditions found in a freshwater loch in Scotland. In addition, the treatment and ultimate disposal of aquaculture effluents was assessed by means of anaerobic biodegradation. Key elements to the viability of such a treatment method were organic waste stabilisation, pathogen destruction and economic feasibility. For the true sustainable development of the aquaculture industry, both environmental and economic criteria must be satisfied. Hence, all aspects of this research were carried out

under the concept of BATNEEC (Best Available Technology Not Exceeding Excessive Cost).

Chapter 3

Materials and Methods

The material and methods required for the design and operation of both the subcage collection system and bench scale anaerobic digesters are detailed in this chapter. This includes design and monitoring of the pilot collection systems through laboratory analysis and field work, and experimental procedures and analysis of treatment methods for the stabilisation of aquaculture waste effluents.

3.1. WASTE COLLECTION SYSTEM

The feasibility of using a sub cage waste collection system was evaluated through a combination of laboratory and field studies for the design and monitoring of the system.

3.1.1. Design of Subcage Waste Collection System

3.1.1.1. Particle size analysis of aquaculture effluents

Samples of waste material from varying sizes and types of salmonids were analysed to determine particle size distribution. Waste matter from rainbow trout (*O. mykiss*, mean individual weight (MIW) = 0.04 kg), and brown trout (*S. trutta*, MIW = 0.35 kg) were obtained from the outlets of two experimental fish tanks in the Department of Biological Sciences at Heriot-Watt University. The tanks were 1.5 m long × 1.5 m wide × 0.6 m deep. Tanks had a stocking density of 1.5 kg m^{-3} and 3 kg m^{-3} for *O. mykiss* and *S. trutta* respectively. Fish in both tanks were fed *ad libitum* with standard diet Trout Rapid 3.5 (Trouw Aquaculture, UK) and Trout Rapid 5.0 (Trouw Aquaculture, UK) respectively.

Each tank received direct water inflow and drained through a standpipe located in the centre of the tank. The standpipe also facilitated solids removal. Triplicate waste sludge samples were collected in 1 l plastic containers from each tank over a three day period in July 1998. These samples were passed through 2,000, 1,000 and 500 μm sieves made from nylon mesh. The material collected on the sieves and the water/particulate mixture passing through the 500 μm mesh was oven dried at 105°C for at least 24 h, until a stable weight was obtained. The proportion of each size fraction (including the <500 μm fraction) was determined as percent dry weight.

The particle size distribution of the <500 μm fraction was determined using a Laser Master Sizer (Malvern Instruments™, UK) fitted with a focal length of 300 mm, capable of measuring particles in the 1.2 – 600 μm range. This instrument determines particle size by laser diffraction. The principle of the method is that a particle passing through the laser beam scatters light at an angle inversely proportional to the size of the particle. The resultant pattern is measured by a photodetector, the signal of which is then processed to calculate the size distribution of particles within a given sample. The sizer was operated in "easy" mode, using deionised water as the background control. Nine replicates per sample were used for each analysis. The particle size distribution of the <500 μm fraction of waste material from the sub cage collection system was also determined using three replicates per sample for each analysis.

3.1.1.2. Net configuration

Net configuration experiments were conducted using a variety of net mesh sizes and types. The nets used were a 6 mm nylon net (hereafter referred to as NY), a 2 mm wind shear (hereafter referred to as WS) net and a 1.5 mm synthetic net (hereafter referred as SN). The physical testing of the net materials was conducted in a sedimentation column

(1.4 m in length and 0.14 m internal diameter). The column was adapted so that sample nets could be set at varying angles of repose (0°, 15°, 30° and 45°) and tested for the capture of suspended waste matter falling through the water column. The sedimentation column was secured vertically, filled with tap water and the angled nets were placed 0.5 m below the water surface (Figure 3.1).

The waste used in the experiment was taken from tanks as described in *Section 3.1.1.1.* A known weight (*c.* 5 g) of mixed wet faecal material was introduced to the sedimentation column just below the surface. Waste was allowed to sediment for *c.* 30 minutes and faecal matter captured on the net was then removed by siphoning and reweighed. Water content of the faecal matter (before and after sedimentation) was determined by placing samples on a preweighed evaporating dish, drying at 105°C until a stable weight was obtained and calculating the weight loss. Triplicate samples of faecal matter were analysed for each angle of repose.

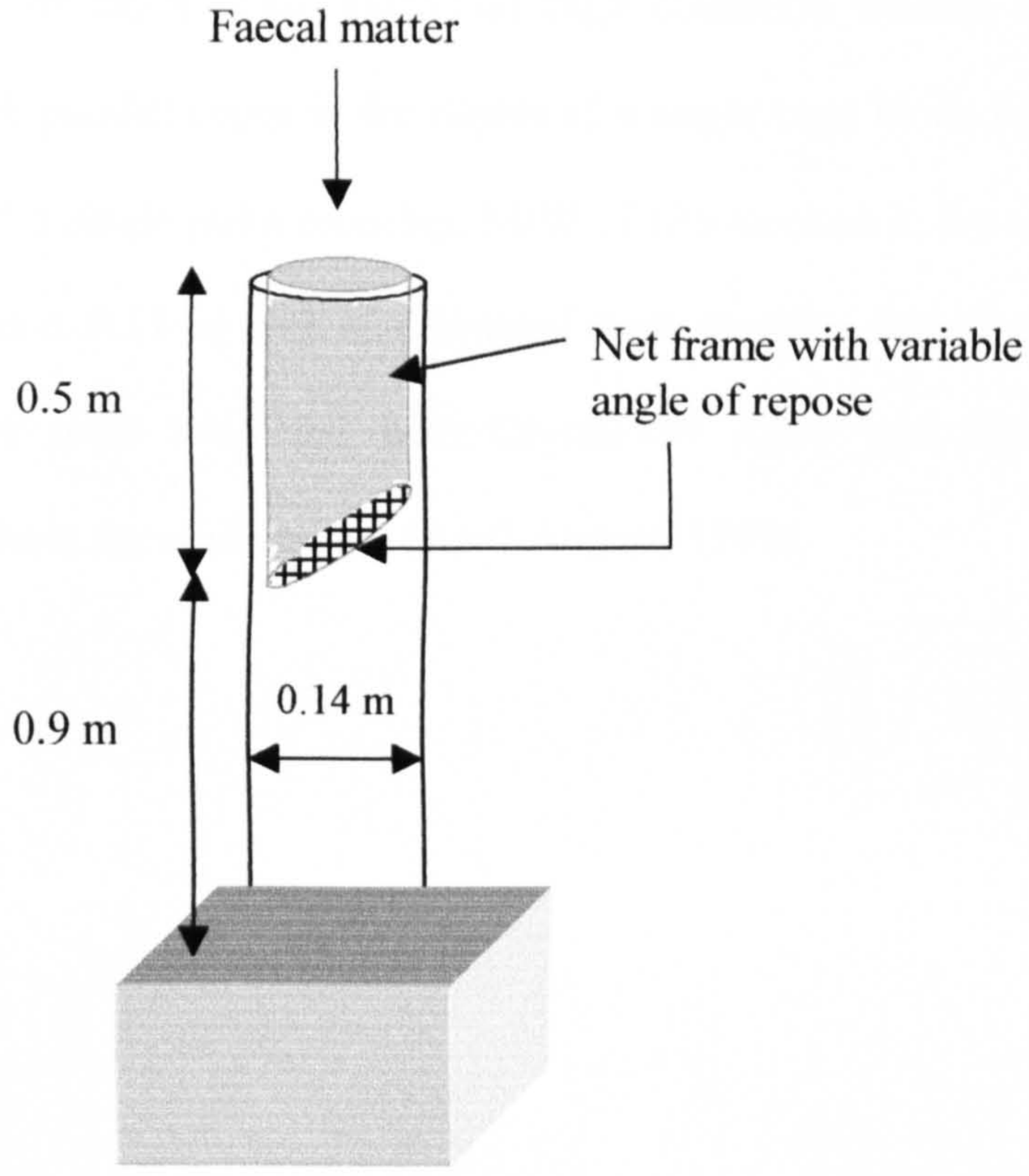


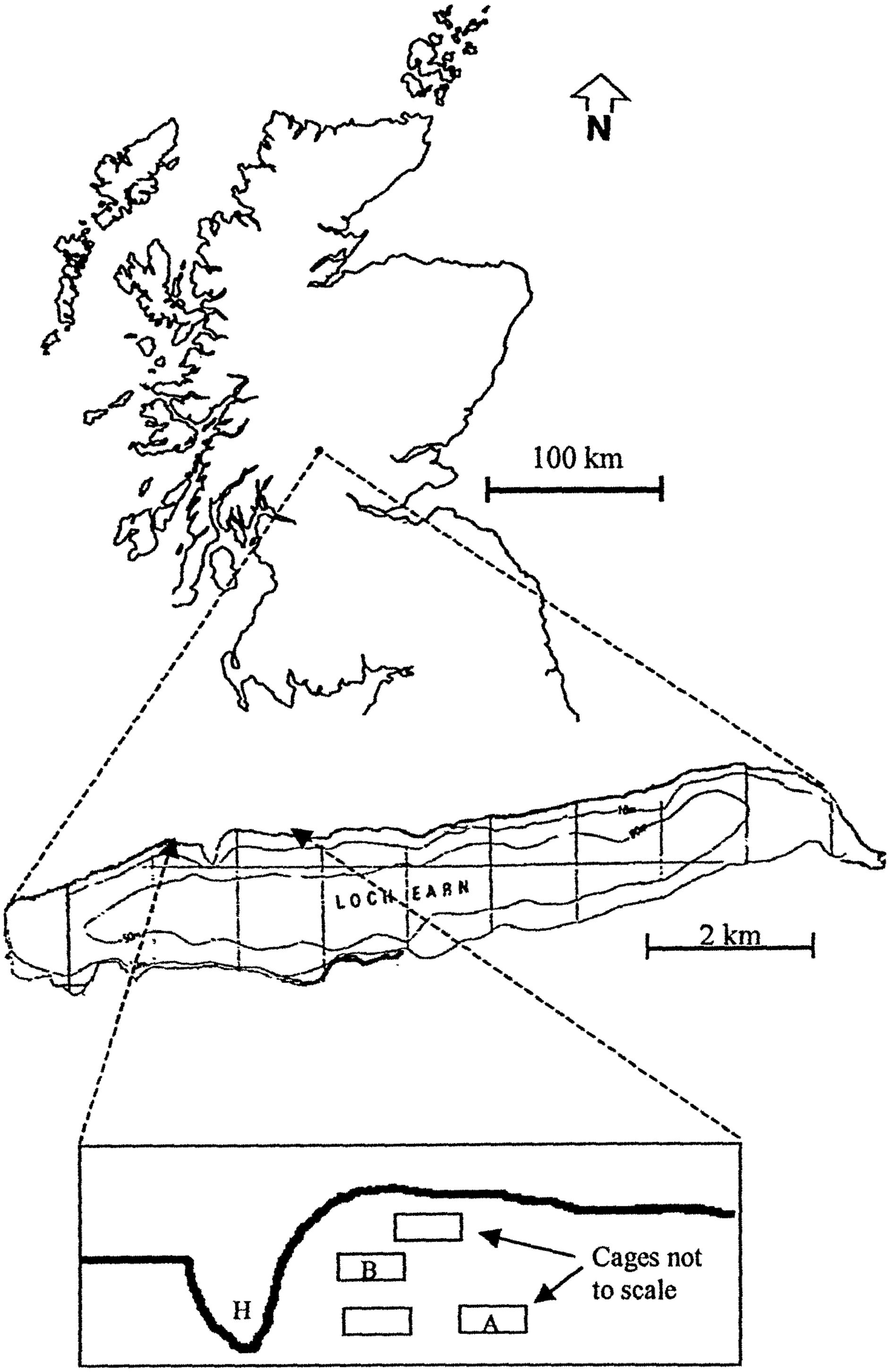
Figure 3.1. Sedimentation column with net frame for net configuration experiment.

3.2. FIELD WORK

3.2.1. Study Site

The study site was situated in Loch Earn, a relatively large (surface area *c.*1 × 10^7 m^2) freshwater lake in central Scotland with an estimated volume of 4.1 × 10^8 m^3 (Murray and Pullar, 1910). The cage farm, situated in an inlet bay within Loch Earn (Figure 3.2), has an annual *O. mykiss* production of *c.* 300 t. Cages were grouped in blocks of twelve with either static or single point moorings, around which they could move freely. Cage dimensions were 6.5 m long × 5.5 m wide × 9 m deep. The fish were fed through a combination of automatic feeder and hand feeding, although hand feeding is the primary

method for the majority of cages. Two individual cage collection devices were each placed directly underneath parallel cages in the centre of a single cage block (cage block A, Figure 3.2), which had a single point mooring. MIW of fish stocked in the cages used at the start of the trial was *c.* 0.11 kg with an estimated mean stocking density of 13.9 kg m^{-3}. Fish in both cages were hand fed with Crystal 50 AX-4 mm diet (Trouw Aquaculture, UK) throughout the study period (April-August, 1999).



H = headland
A = Swinging cage block
B = Static cage block

Figure 3.2. Location of study site, at Loch Earn, Scotland.

3.2.2. Installation of Subcage Collection Systems

Each collector consisted of a fine meshed netting attached to the base of the original cage netting using industrial cable ties for ease of removal (Figure 3.3). Weighted ropes were used to give the collector a cone shape appearance and maintain the angle of repose. At the base of the collector netting, a 90 l solid funnel connected to a 10 cm diameter flexible hose was used to remove solid wastes (uneaten feed, faeces and fish mortalities) to a container at the surface. An airlift system was employed to achieve this. Compressed air was injected into the effluent hose 6 m below the water surface causing liquid in the hose to become less dense and thus to rise. Dead fish were separated from the effluent stream using a large meshed net. Initial problems with the hosing not remaining vertical and thus hindering the airlift system were overcome using an easily removable light metal frame to keep the piping rigid. A waste removal flow rate of 9 - 10 l s^{-1} was achieved using a compressor with a flow capacity of 0.020 m^3 s^{-1} (1 atm). Two collection systems were installed with varying net sizes for comparison, a wind shear net and a 6 mm nylon net. Waste material was pumped from the collection systems on a weekly basis by farm staff. Another cage within the same cage block with no collector attached was used as a control for water quality sampling.

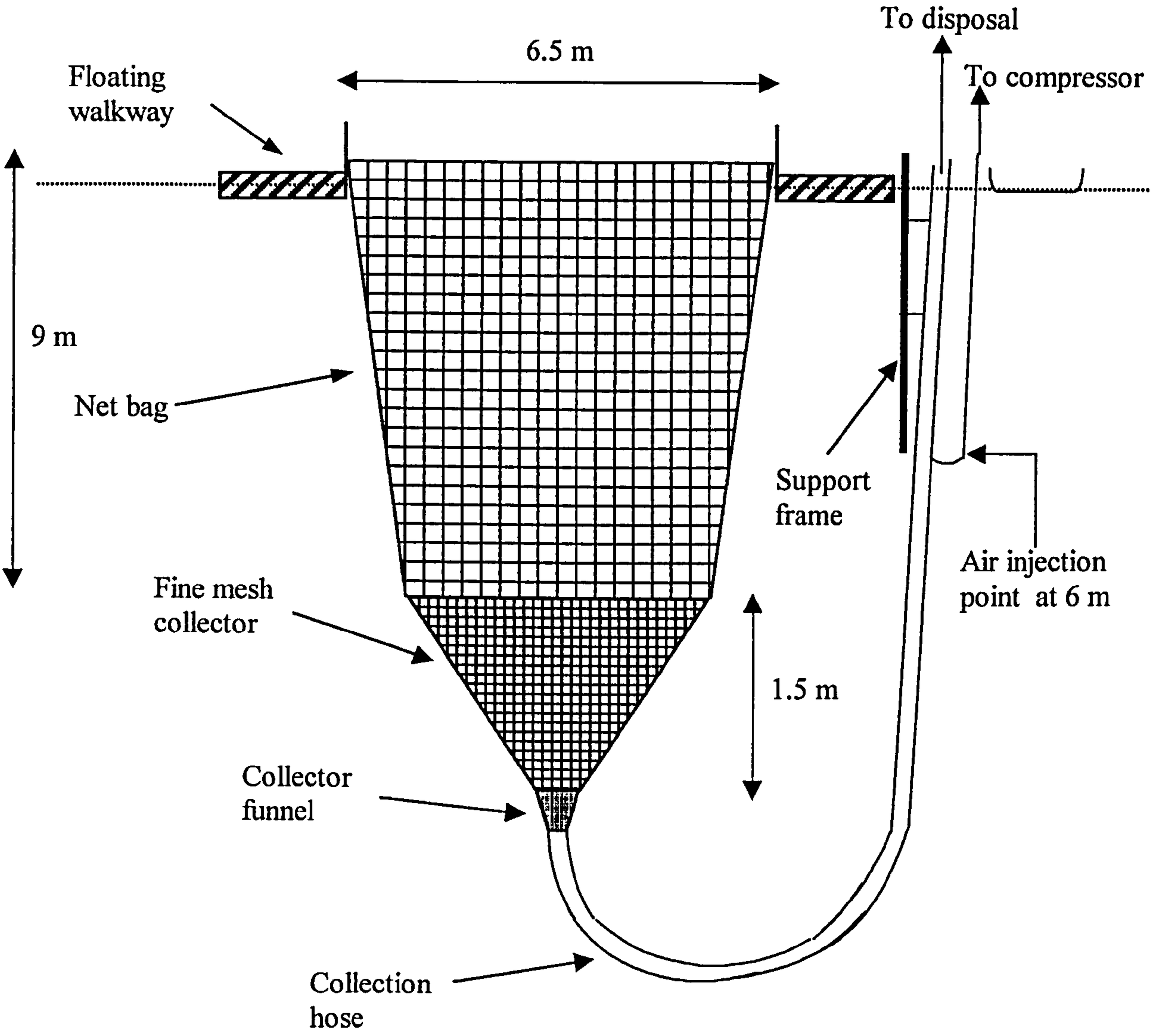


Figure 3.3. Schematic of the waste collection system installed at Loch Earn (Not to scale).

3.2.3. Meteorological Data

A Skye DataHog 2, model SDL 5000 (Skye Instruments Ltd., UK), meteorological station was used to collect data at the survey site. The weather station was deployed on the single point mooring cage block (cage block A, Figure 3.2) containing the two waste collection devices. It consisted of an electronic data logging system integrated with two temperature sensors and a wind run sensor, from which wind speed could be calculated. Surface water (~ 0.5 m depth) and air temperature (~ 1 m above water surface) were measured using two separate thermistors (Model SKTS 200, Skye Instruments Ltd., UK). Wind run was determined using a Thies-Clima Anemometer (Adolf Thies GmbH, Gottingen, Germany) installed approximately 2.5 m above the water surface. It was not possible to determine wind direction due to cage motion around the single point mooring of the cage block. Weather data was sampled for 20 minutes and logged at 2 h intervals. Data recorded by the logger was downloaded to a lap-top computer via a built in RS232 interface in the logger. The meteorological station was deployed concurrently with the installation of the waste collection systems (April-August, 1999).

3.2.4. Sediment Traps

The dispersal and sedimentation of material from the fish cage was estimated using sediment traps. An array of cylindrical sediment traps mounted on a stainless steel frame was deployed in triplicate around the periphery of a single cage with collector (Figure 3.4). Traps were constructed from PVC tubing (internal diameter 5 cm and height 30 cm, Elberizon, 2000) with an aspect ratio (height:diameter) of 6:1, as recommended by Reynolds (1979). Triplicate sediment traps were deployed for 24 h at the midway point on each side of the cage at depth intervals of 0.5, 4.5 and 8.0 m. Sediment trap frames at

varying depths on each cage side were connected in series using nylon rope. Waste dispersion was measured in g m^{-2} d^{-1} as dry matter.

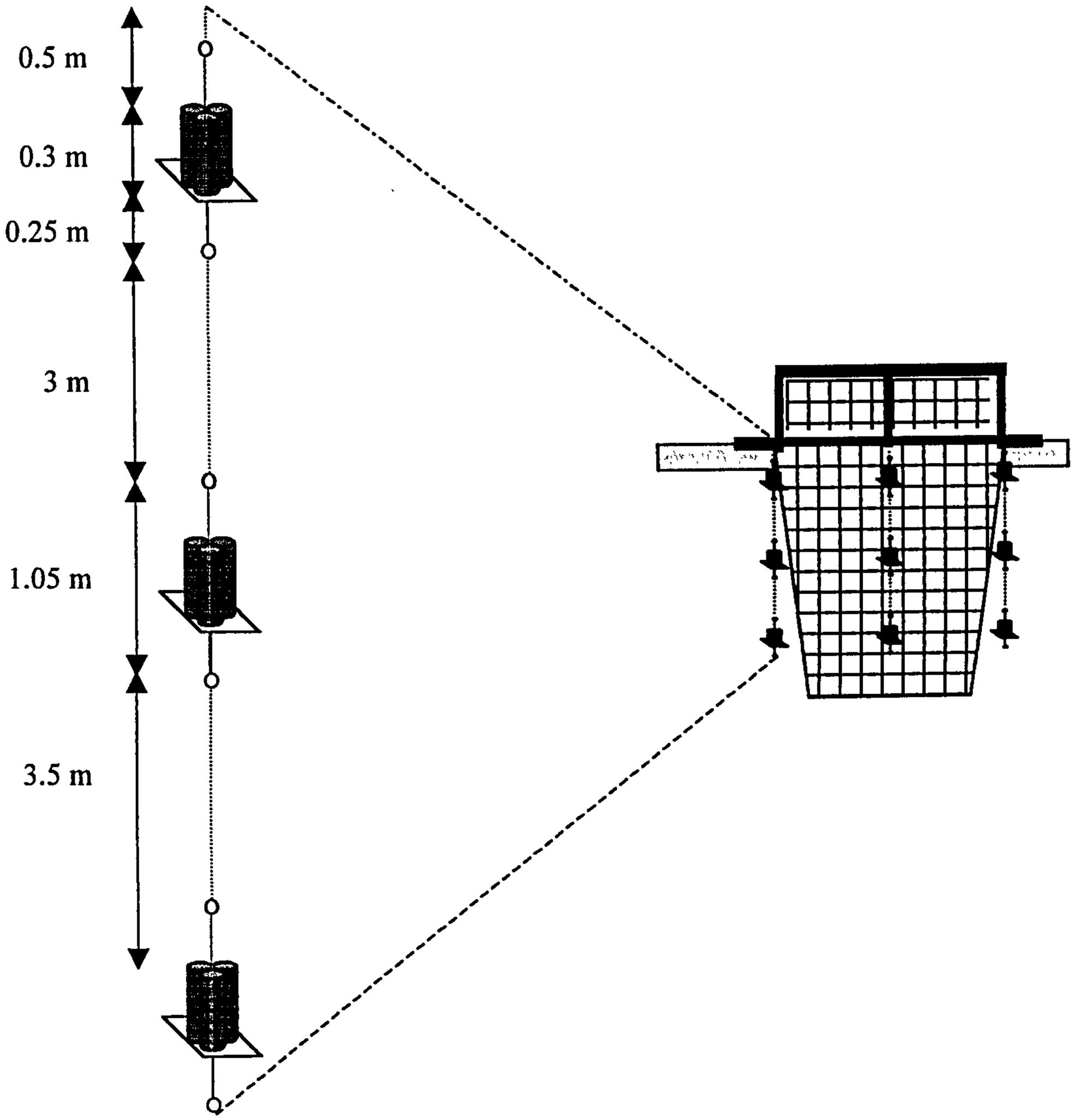


Figure 3.4. Sediment trap arrangement around the periphery of the cage.

3.2.5. Sampling and Analysis

Waste effluent samples from the collection system were taken and TS, SS, C, N and P content of the solids collected analysed as described below (see water [*Section 3.2.5.1*] and solids [*Section 3.2.5.1*] analyses). Fish and feed samples were taken at monthly intervals and examined for the above. DO, N and P in the water column throughout the cage depth profile were also examined and compared to that of a control cage which had no collection system. All laboratory water and solid analyses of cage water quality and subcage waste collector samples were carried out by the Institute of Aquaculture, University of Stirling, UK.

3.2.5.1. Water analyses

A YSI model 57 meter with a temperature sensor and polarographic oxygen electrode on a 10 m cable was used to record temperature and dissolved oxygen profiles at the cages. Using a self-closing vanDorn sampler, water samples were taken at surface and at 8 m depths inside the cage. Samples were stored in polyethylene bottles and transported to the laboratory in darkness at 4°C, before processing. Sub-samples were decanted for pH, conductivity, alkalinity (Alk) and TP analyses. The remaining samples were filtered through 1.2 μm Whatman GF/C filter papers. The filtrate was stored frozen for later analyses of dissolved reactive P, and ammoniacal nitrogen, nitrate and nitrite (all determined as N).

Water sample pH values were recorded using a Philips PW9409 meter and combination glass electrode. Conductivity measurements were made with a HACH44600 conductivity/TDS meter and probe. Alk levels were determined by titrimetric methods (Mackereth, 1963). Following sulphuric acid/potassium persulphate digestion, total P was

measured by spectrophotometer as dissolved reactive P, using a molybdenum blue complex technique (HMSO, 1981).

Ammonia (TAN), NO_2^- and NO_3^- concentrations were determined simultaneously using a triple channel Technicon II Autoanalyser. Ammonia was quantified by salicylate/hypochlorite chemistry (Method F; HMSO, 1982). Nitrate and nitrite were determined using the same NED/sulphanilamide chemistry, following reduction of nitrate to nitrite by cadmium catalysis (Method D; HMSO, 1982).

3.2.5.2. Solids analyses

The SMP (Standard Method Procedure) for paper filtration (HMSO, 1980) was carried out to determine rate of solid deposition in sediment traps. Samples were passed through pre-washed and pre-weighed 1.2 μm, Whatman GF/C filter papers, which were then redried at 105°C for 24 h and until a stable weight was obtained and reweighed. Where there was excessive material, samples were instead placed in evaporation dishes and oven-dried as above. Solid mass was calculated based on the estimated weight loss.

Fish, fish feed and cage waste were all analysed for P, N and C contents. Duplicate sample replicates were oven-dried at 105°C for 24 h to obtain a stable dry weight. TP was determined by digesting four 15 mg sub-samples with nitric acid, followed by 70% perchloric acid (Allen, 1974). As these two digestion steps were effective against the largely organic matrices of feed, fish and waste, the sulphuric acid digestion step was not necessary. Following pH correction (Strickland and Parsons, 1972), TP was measured by spectrophotometer as dissolved reactive P, using a molybdenum blue complex technique (HMSO, 1981). P concentrations were expressed as mg P g^{-1} dry matter. C and N content of samples were determined in duplicate by a Perkin Elmer Series II CHNS/O analyser.

The sample weight required for each analysis was 10 - 20 mg of oven dried matter. C and N results were determined as percent dry weight values.

3.2.6. Water Current Velocity and Dispersion Coefficient Measurements

The measurement of water current speeds and dispersion coefficients at the study site was carried out by Dunstaffnage Marine Laboratory (DML), Oban, Scotland (Cromey and Provost, 2001).

The measurement of water current velocities was achieved by means of three different methods. An Acoustic Doppler Current Profiler (ADCP) (model 300 KHz RDI Sentinel Workhouse) was deployed in parallel with rotary Aanderaa current meters (Designed and manufactured by the Marine Technology Group at DML). ADCP instruments use the Doppler effect by transmitting sounds at a fixed frequency and listening to echoes returning from sound scatterers in the water column (e.g. plankton and suspended sediment). The ADCP unit is more commonly used in the marine environment and therefore its accuracy in freshwater was verified by using the Aanderaa meters. However, the rotary nature of these meters means some inertia is required to start the rotor spinning (*c.* > 3 cm s^{-1}), therefore low current velocities will not be measured by this method.

Furthermore, differential geographical positioning system (DGPS) drifter buoys (Designed and manufactured by the Marine Technology Group at DML) were deployed to enable the determination of horizontal diffusivity in the water column where diffusivity is related to the change in variance of the spatial distribution of the drifter buoys over time. The velocity of the drifters may also be used to compare the accuracy of both methods described above.

For the comparison of water current velocity with wind speeds, wind data was recorded using a meteorological station as described in *Section 3.2.3.* A second meteorological station was deployed on an inner static block of cages (cage block B, Figure 3.2) to allow for mean hourly measurements of wind direction. Wind direction was detected using a wind vane (Resitor W200P, Skye Instruments Ltd., UK).

The ADCP was mounted on a trawl proof frame on the loch bed and set up to record the current velocity every 10 minutes at two metre intervals for the entire depth profile of the water column. The instrument was deployed on 20.03.01 in 36 m depth and recovered on 22.03.01. On 21.03.01, DGPS drifters were used in the area around the ADCP as further verification of the other methods described above. The instrument was then redeployed for the remainder of the study period (04.04.01) in 38 m depth. The DGPS drifter survey was carried out on two different time periods, initially on 21.11.00 and repeated on 21.03.01.

3.3. AQUACULTURE WASTE EFFLUENT TREATMENT

3.3.1. Laboratory Scale Anaerobic Digesters

The laboratory scale anaerobic digesters were constructed using glass aspirators of height 0.25 m and internal diameter 0.17 m (Figure 3.5). The working volume of each reactor was 4 l with a headspace volume of approximately 1 l. Agitation was achieved using a single blade impeller (length 70 mm and height 10 mm) attached to a stainless steel shaft driven by an IKA RW 20 laboratory stirrer motor at a rate of 100 rpm (mixing rate based on that of previous studies using agricultural sludge, Kiely, 1997). The mesophilic and thermophilic digesters were maintained at 35°C and 55°C respectively by means of external copper heating coils, through which warm water was circulated using Grant FH-

15A flow heaters. Bubble packing was wrapped around these digesters for thermal insulation. The psychrophilic reactor was unheated, but was insulated with bubble packing to minimise temperature fluctuations. Temperature was therefore maintained at room temperature (18-22°C).

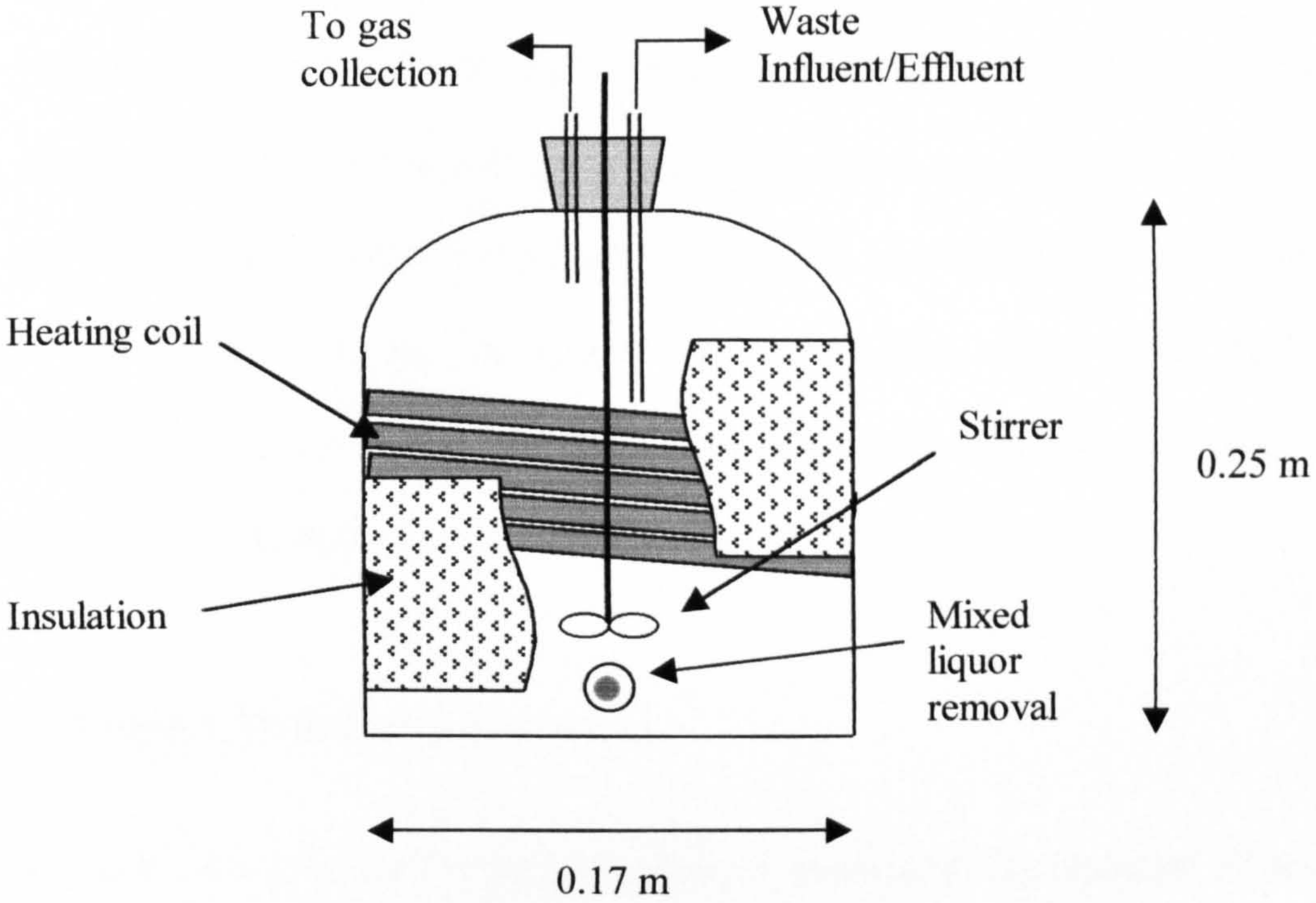


Figure 3.5. Schematic of a laboratory scale anaerobic digester.

3.3.2. Digester Start-Up and Feeding Procedure

The anaerobically digested sewage sludge used in the start-up of the digesters was obtained from the Bathgate municipal treatment works operated by West Lothian Council. The sludge was passed through a plastic sieve of pore size approximately 1 mm prior to use to remove any large solids present.

Feed material was obtained from the Buckieburn land based fish farm used for research by the University of Stirling's Institute of Aquaculture. It was captured in a 100μm net, which allowed for preliminary dewatering. The collected waste was then allowed to settle and excess water was removed by siphoning. When feeding the digesters, the stirrers were turned off and the digester contents allowed to settle for *c.* 30 minutes. A known quantity of liquor was removed through a combined influent/effluent port at the top of each digester by means of a peristaltic pump (model Watson Marlow 505) then an equivalent volume of fresh feed was added via the same port, *e.g.* at a HRT of 23.33 days, 400 mls of liquor was removed, and replaced with 400 mls of fresh feed three times per week. The stirrers were then reengaged. Raw aquaculture wastes from Buckieburn generally had pH values in the range 5.8 - 6.6, which were adjusted to around 7.0 by addition of sodium hydrogen carbonate prior to use in the digester.

3.3.3. Biogas Collection and Analysis

Biogas production rates were measured daily by downward displacement of acidified water in a collection vessel. The biogas was collected under ambient conditions, *c.* 20°C and 1 atm. Biogas measurements were not taken during digester feeding to avoid errors due to displacement of the headspace biogas. The gas line between the digester and collector was fitted with a sampling port for analysis of biogas composition, which was monitored using a Perkin Elmer Gowmac Series 600 gas chromatograph fitted with a Porapak Q column and a thermal conductivity detector (TCD). The column was of stainless steel construction and was 2 m in length with an internal diameter of 2 mm. The injector, oven and detector temperatures were 90°C, 60°C and 120°C respectively, while the helium (BOC gases) carrier gas was flowing at 30 ml min^{-1}.

3.3.4. Feed, Digester Liquor and Effluent Sampling and Analysis

TS, SS, COD and BOD were monitored on a weekly basis once the digesters were stabilised. Mixed digester liquor TS and SS were determined approximately once every 2 weeks. Effluent Alk and VFA concentrations were analysed weekly and more frequently during periods of acclimation.

COD was measured by the small-scale closed tube digestion method described by HMSO (1986). Soluble COD was determined by filtering digester effluent samples through 0.45 μm Whatman cellulose nitrate filters prior to analysis. BOD was determined by a Hach 2173B manometric BOD apparatus.

Total ammonia nitrogen (TAN) and reactive P were measured colorimetrically by a Hach DR/2000 spectrophotometer. Ammonia was quantified using salicylate/hypochlorite chemistry (Salicylate method) and reactive P (orthophosphate) using the molybdenum blue complex technique (Hach Company, 1988). TS, SS and VS were determined as described in Standard Methods (APHA et al., 1995). pH was measured using a Russell CE7L/S7 combination electrode and Corning 113 pH meter. Alk was determined by titration of samples to pH 4.5 with 0.1 M HCl solution, results being expressed as mg $CaCO_3$ l^{-1} equivalents.

VFA concentrations were measured with a Perkin-Elmer 8500 gas chromatograph equipped with a stainless steel column of internal diameter 2 mm and length 2 m, packed with 10% free fatty acid phase on 80/100 mesh Chromosorb W-AW (Supelco, Bellefonte, PA, USA). Compounds leaving the column were detected by flame ionisation detector (FID) and the peak area determined by JCL6000 integration software (Jones Chromatography Ltd., Hengoed, Mid-Glamorgan, UK). Injector and FID temperatures

were both 220°C. Oven temperature was held at 150°C for 1 min at the start of each analysis, then increased to 170°C over 2 min and held there for a further 7 min. N_2 (BOC gases, O.F.N. grade) carrier gas flow was 20 ml min^{-1}. Samples were prepared for analysis by filtration through 0.45 μm Whatman cellulose nitrate filters, followed by acidification with 1 part formic acid to 10 parts sample.

3.4. POST-TREATMENT OF ANAEROBICALLY DIGESTED AQUACULTURE EFFLUENTS

3.4.1. Biofiltration Unit

The biofilter unit consisted of a cylindrical perspex packed fixed bed with plastic media and a separate collection basin for recycle and treated effluent (Figure 3.6). The biofilter had a total volume of 0.0024 m^3 with a collection basin volume of 0.00046 m^3. Digester effluent was applied to the filter media by means of a fixed distributor cross arm. The filter medium was composed of 0.015 m pall rings.

The biofilter was fed the wastewater from the digester operating thermophilically on aquaculture effluents, operating at a HRT of 23.33 days and with an organic loading of *c.* 0.34 kg COD m^{-3} day. The digester effluent was stored in a freezer until required. It was allowed to defrost for 24 hrs before use. The digester effluent, stored in a continuously stirred vessel, was then supplied continuously to the filter media via a peristaltic pump (model Watson Marlow 505). To ensure sufficient wetting of the media, a recycle stream comprising fresh waste and oxidised waste, was employed at flow rate of 100 l day^{-1}.

3.4.2. Sampling and Analysis

The performance of the biofilter was monitored twice weekly from the analysis of both inlet and outlet samples for COD, TS, SS, TAN, reactive P and pH were analysed as described in *Section 3.3.4*. In addition, NO_2-N and NO_3-N were determined colorimetrically with a Hach DR/2000 spectrophotometer using the same NED/sulphanilamide chemistry (Diazonium method), following reduction of nitrate to nitrite by cadmium catalysis (Hach company, 1988).

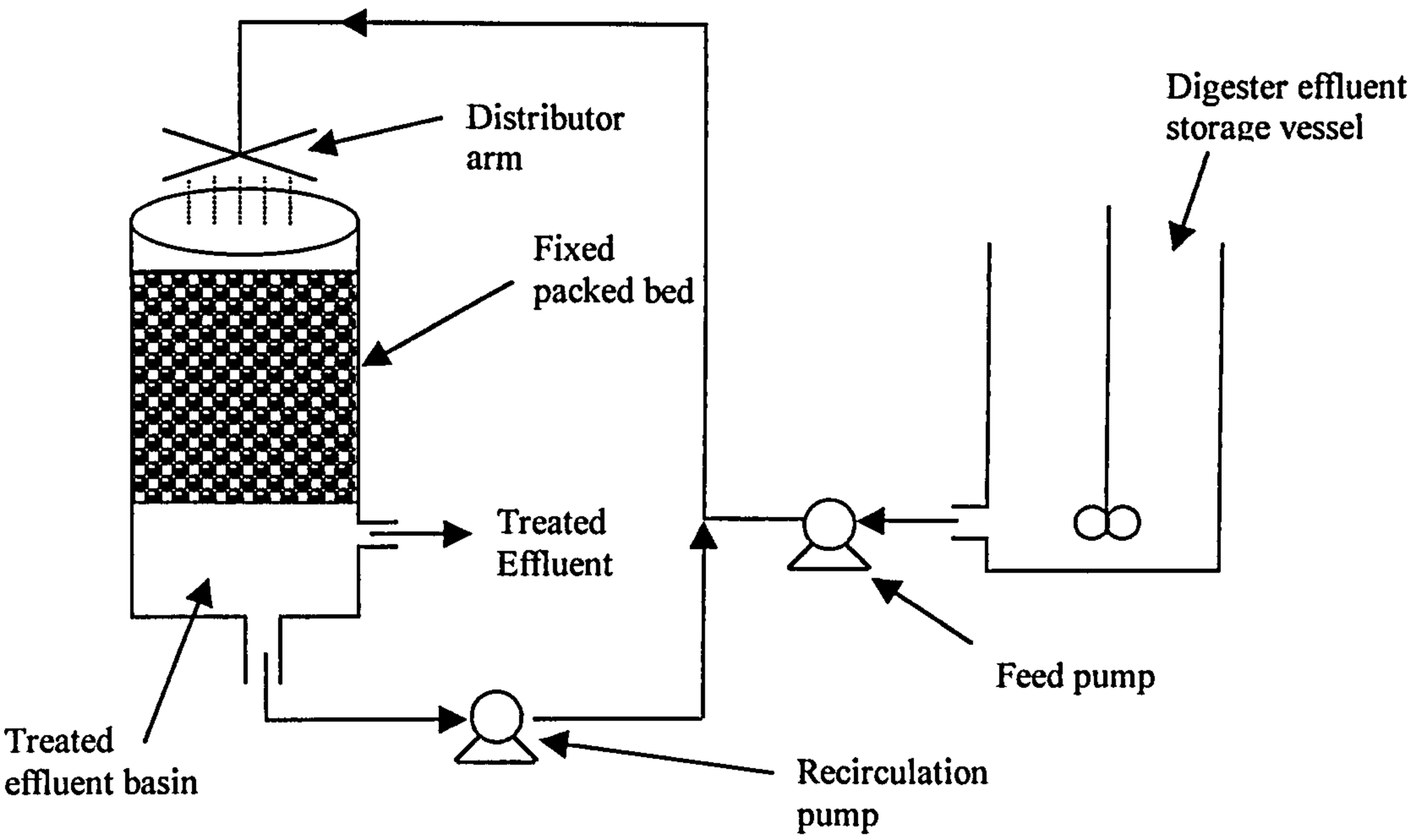


Figure 3.6. Schematic of biofiltration unit and feed vessel.

3.5. PATHOGEN INACTIVATION

The objective of this study was to evaluate the pathogen reduction performance of anaerobic digestion operating at both thermophilic and psychrophilic temperatures. A common fish bacterial disease, enteric redmouth (ERM) was chosen to carry out the study. It was not possible to investigate the destruction of viruses due the requirement of specialist equipment for containment and health and safety risks.

3.5.1. Enteric Redmouth Disease.

Enteric redmouth disease (ERM) is a systematic bacterial infection of fish but is mostly restricted to salmonids principally *O. mykiss.* It was first described by Rucker (1966) after an outbreak in the Hagerman Valley, Idaho, USA and subsequently named *Yersinia ruckeri* by Ewing et al. (1978). The organism is a member of the *Enterbacteriaceae* family and comprises a homogenous group of Gram-negative, slightly curved rods of 1.0 × 2.0 – 3.0 μm in size, which are motile usually by means of seven or eight peritichously arranged flagella. Three serotypes have been identified with the most virulent being Type 1, the Hagerman strain with an LD_{50} dose of 3.0×10^5 cells ml^{-1}. *Y. ruckeri* most commonly affects fish of approximately 7.5 cm in length with severity peaks at a water temperature of 15 – 18°C (Austin and Austin, 1992, 1999). Typical symptoms of the disease are reddening in the mouth and throat caused by subcutaneous haemorrhaging. This is accompanied by darkening of the skin, inflammation and erosion of the jaw and palate, haemorrhaging around the base of the fins and the infected fish are observed to be lethargic and anorexic (Busch, 1983; Austin and Austin, 1992, 1999). *Y. ruckeri* commonly causes sustained low level mortality, eventually resulting in high losses.

The pathogen may be readily recovered from the kidney on routine bacteriological media, e.g. tryptone soya agar (TSA) or brain heart infusion agar (BHIA), following incubation at 20 - 25°C for 48 h whereupon round raised entire shiny off white colonies of 2 - 3 mm diameter develop. Three selective media have also been devised namely Waltman and Shotts, (1984), Rodgers (1992) and Furones et al. (1993) (Austin and Austin, 1999). For this investigation, ribose ornithine deoxycholate (Rodgers, 1992) selective medium was used.

3.5.2. Preparation of Bacteria and Culture Media

Ribose ornithine deoxycholate medium (Rodgers, 1992) was used as a selective medium for the growth of *Y. ruckeri* (Table 3.1). The solution was adjusted to pH 7.4 and autoclaved at 121°C for 15 minutes. After cooling (<50°C), 10 ml of filtered (0.22 μm) solution containing 0.5 g of sucrose ml^{-1} was added to the basal medium. The medium was then allowed to set for 48 hours. Type 1 *Y. ruckeri,* obtained from the Department of Biological Sciences, Heriot-Watt University, was used in the experiment and bacterial numbers were determined using a haemocytometer and an Olympus CH2 microscope in conjunction with a viable plate count.

Table 3.1. Composition of ribose ornithine deoxycholate medium (Rodgers, 1992) for the isolation of *Yersinia ruckeri* (Austin and Austin, 1999).

Composition	Concentration (% w/v)
Yeast extract	0.30
Sodium deoxycholate	0.10
Sodium chloride	0.50
Sodium thiosulphate	0.68
Ferric ammonium citrate	0.08
Maltose	0.75
Ribose	0.38
Ornithine hydrochloride	0.38
Sodium dodecyl sulphate	0.10
Phenol red	0.01
Agar	1.25

3.5.3. Sample Analysis for Pathogen

Sample volumes of 0.5ml were plated in duplicate onto the selective media (as described in *Section 3.5.2*) and allowed to incubate at room temperature for 48 h. Inoculated plates were examined for the presence of *Y. ruckeri* and the number of colonies was recorded.

3.6. ESTIMATION OF ULTIMATE CH_4 YIELD AND KINETIC COEFFICIENTS

The rate of substrate utilization was determined by direct association with CH_4 production as discussed in *Section 2.7*. A slightly modified version of the biochemical methane potential (BMP) assay developed by Owen et al. (1979) was used for the determination of ultimate CH_4 production and hence the rate of substrate degradation. The assay was designed to assure that the degradation of a compound is not limited by nutrients, inoculum, substrate toxicity, pH, oxygen or substrate overloading. Stock solutions are prepared and blended to make up a defined media to meet these requirements.

3.6.1. Preparation of Media

Concentrated stock solutions (Table 3.2) were used for the prepartion of the defined media and stored at 4°C. In addition to 1 l of deionised water, 5.4 ml of S2 and 27 ml of S3 were added to a 2 l volumetric flask. The flask was filled to the 1.8 l mark with deionised water and boiled for 15 minutes while under a continuous atmosphere of N_2 gas at a flow rate of approximately 1 l min^{-1}. After cooling to room temperature, 1.8 ml of S6, S4 and S5 were added to the flask. The flushing gas was changed to a 30% CO_2, 70% N_2 mixture and 8.4 g of $NaHCO_3$ was added to the flask. Gas was continuously flushed through the media in the volumetric flask until the pH of the solution stabilised at 7.1. The flask was then sealed, while minimising the introduction of air to the vessel.

Table 3.2. Stock solutions for preparation of defined media.

Solution	Compound	Concentration ($g\ l^{-1}$)
S1	Sample	See *Section 3.6.3*
S2	$(NH_4)_2HPO_4$	26.7000
S3	$CaCl_2.2H_2O$	16.7000
	NH_4Cl	26.6000
	$MgCl_2.4H_2O$	120.0000
	KCl	86.7000
	$MnCl_2.4H_2O$	1.3300
	$CoCl_2.6H_2O$	2.0000
	H_3BO_3	0.3800
	$CuCl_2.H_2O$	0.1800
	$Na_2MoO_4.H_2O$	0.1700
	$ZnCl_2$	0.1600
S4	$FeCl_2.4H_2O$	370.0000
S5	$Na_2S.9H_2O$	500.0000
S6	Biotin	0.0028
	Folic acid	0.0020
	Pyridoxoxine hydrochloride	0.0100
	Riboflavin	0.0051
	Nicotinic acid	0.0059
	Thiamin	0.0054
	Pantothenic acid	0.0055
	B_{12}	0.0001
	p-aminobenzoic acid	0.0050

3.6.2. Preparation of Assay Bottles

The BMP assay was conducted using 250 ml and 350 ml conical flasks for the psychrophilic and thermophilic assays respectively. The flasks were flushed with a mixture of 30% CO_2, 70% N_2 at a flow rate of 1 l min^{-1} for *c.* 5 minutes, then sealed with

rubber bungs and allowed to equilibrate to incubation temperature before introduction of the sample, inocula and defined media. The rubber bungs were altered to allow for the measurement of biogas evolved during the assay (Figure 3.7).

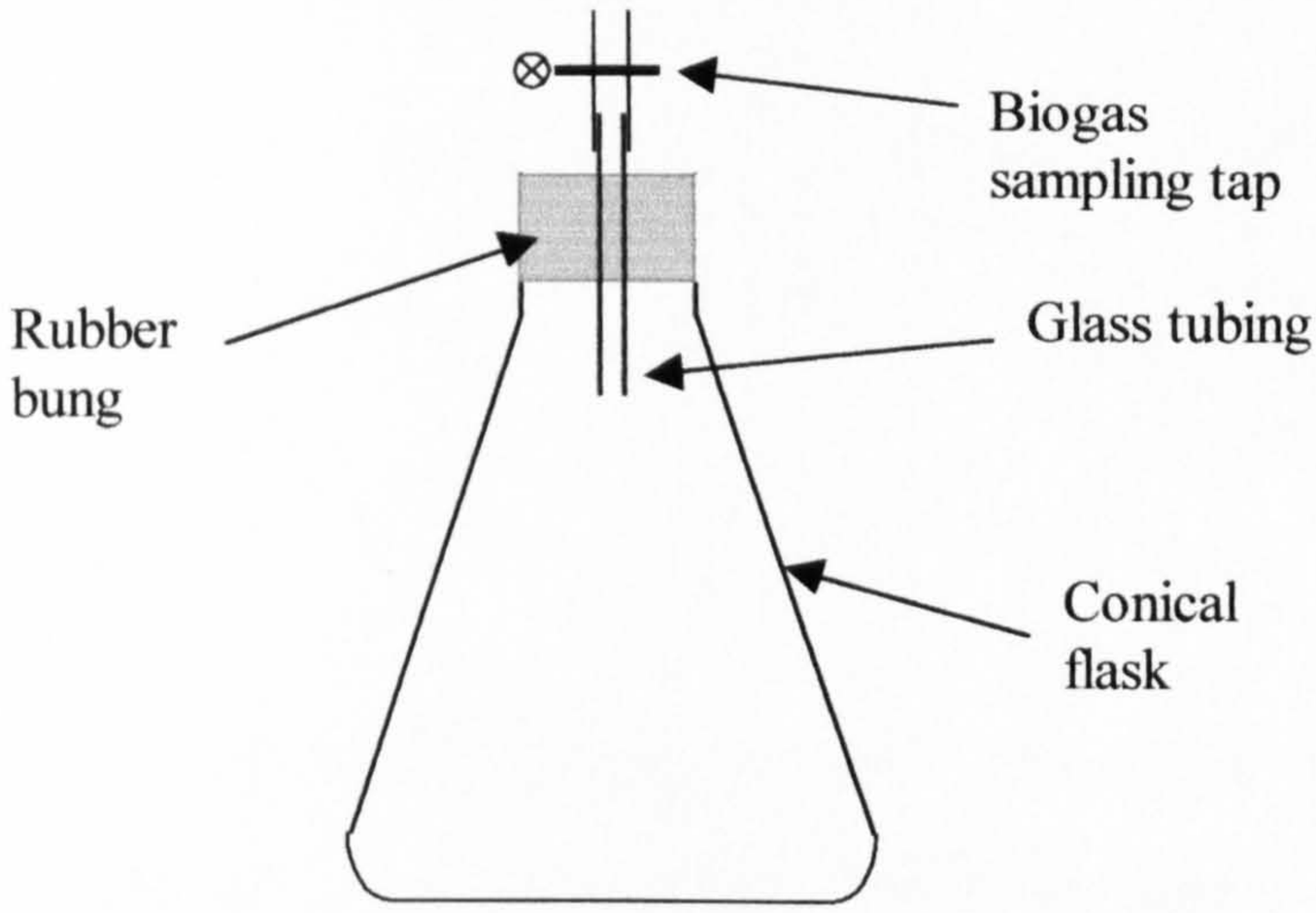


Figure 3.7. Schematic of flask used for the BMP assay.

3.6.3. Preparation of Thermophilic and Psychrophilic BMP Assays

Proper sample size and liquid to void volume (headspace) ratio are important for the precision and accuracy of results in the BMP assay. Owen et al. (1979) recommended the degradable sample COD be *c.* 2 g l^{-1} and the use of a 20% inoculum volume in the assay. The void volume ratio may be adjusted in order to increase the accuracy of the assay.

The inoculum for the assays was taken from the anaerobic digesters as described in *Section 3.3.2*. Both the thermophilic and psychrophilic reactors had a HRT and organic loading rate of 23.33 days and 0.371 kg COD m^{-3} day^{-1} respectively. Defined media and inoculum were introduced separately to the assay flasks whilst under flushing from a 70% N_2, 30% CO_2 gas mixture. In order to determine the overall rate of substrate utilisation, a

variety of sample concentrations were employed, 0.7, 1.7, 2.7 and 4 g VSS l^{-1}. Both sets of assays were accompanied with positive controls of cellulose (6 g l^{-1}) and blank controls containing only inoculated media and deionised water to ensure void volume ratios were similar to the sample assays. The flasks were sealed and placed at the appropriate incubation temperatures. The psychrophilic assays were placed in a 20°C incubator and the thermophilic assays in a 55°C oven. Both samples and controls were assayed in duplicate.

3.6.4. Data Analysis

A detailed account of CH_4 produced during the assay was recorded. After each sampling, the value of the measured CH_4 volume produced was converted to dry gas at 1 atm (Weather Monitor II, Davis Intruments, USA) and 0°C and added to the previous measurements. This cumulative CH_4 volume removed was then added to the CH_4 present in the headspace of the bottle to determine the total cumulative CH_4 volume at the time of sampling. The total cumulative CH_4 volumes were corrected for CH_4 production to the medium and inoculum by subtracting the averaged blank control volumes from each flasks total cumulative CH_4 volume. Finally, the corrected cumulative CH_4 yield was calculated by dividing the corrected volume by the weight of sample VSS added to each flask.

Chapter 4

The Subcage Collection of Aquaculture Waste Effluents

4.1. NET CONFIGURATIONS

The optimum angle of repose was found to be 45° with all three nets, maximum capture being obtained with the SN (Figure 4.1). However, in discussion with farm staff, it was decided that the coarser net mesh (WS) should be used in order to prevent fouling and to ensure adequate water and oxygen transport through the cage. The angle of repose was decreased to 20 – 30° for farm management purposes. For harvesting and grading of fish at the site, cages must be docked at a jetty next to the shoreline. An angle of repose of 45° raised concerns from farm staff regarding sufficient water depth at the jetty point for docking of the fish cage with collection system.

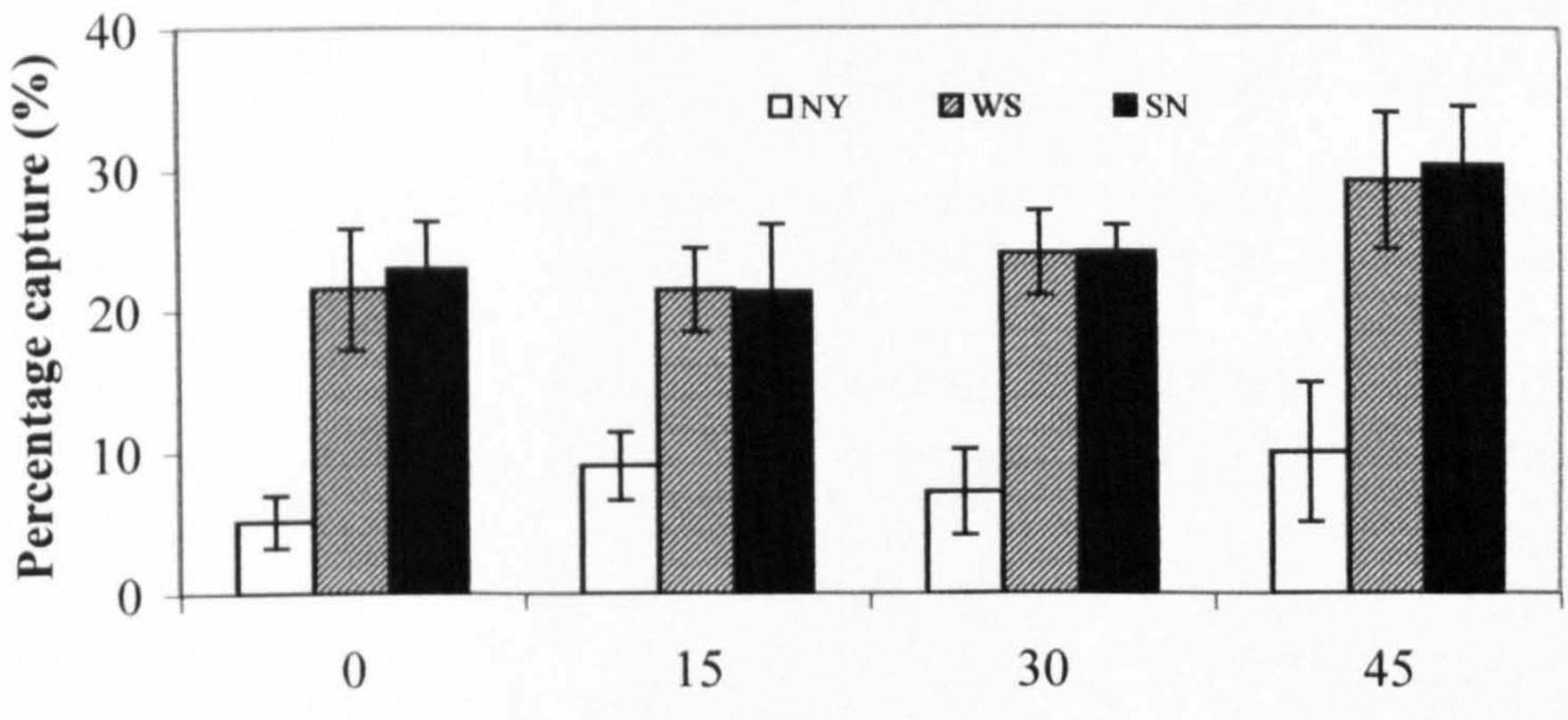


Figure 4.1. Percentage capture of waste material from experimental tanks with varying net material and angles of repose (Mean ± SD, $n = 3$).

4.2. WASTE COLLECTION SYSTEMS

The performance and efficiency of the waste collection systems was monitored from April 1999 to August 1999. Water quality throughout both cages with collection systems and the control cage was monitored monthly. Water quality at the two experimental cages, in terms of N and P present in the water column, was unaffected by the presence of the waste collection systems in comparison with the control cage (Table 4.1). Dissolved oxygen levels were similarly unaffected by the presence of the collector and cone. Mean percentage saturation varied little with depth or cage type, and values remained high throughout the observation period (Table 4.2).

Table 4.1. Mean concentration (mean ± SD, n = 5) of major nutrient parameters within cages fitted with collector (NY and WS) and control, April-August 1999.

Site	Depth (m)	TP	DRP	NO_2	NO_3	TAN
		($\mu g\ P\ l^{-1}$)			($\mu g\ N\ l^{-1}$)	
NY						
	0	12.5 ± 3.1	2.4 ± 0.6	2.6 ± 1.0	203 ± 34.2	50 ± 16.8
	8	11.1 ± 1.7	1.8 ± 0.3	2.5 ± 1.1	200 ± 48.4	46 ± 12.6
WS						
	0	18.7 ± 8.2	2.6 ± 0.9	2.6 ± 1.0	185 ± 56.7	62 ± 28.5
	8	11.6 ± 3.1	1.5 ± 1.1	2.7 ± 1.0	214 ± 71.5	49 ± 16.8
Control						
	0	17.6 ± 11.5	1.2 ± 0.6	2.7 ± 1.1	216 ± 42.3	56 ± 25.7
	8	12.8 ± 1.7	2.4 ± 0.5	2.4 ± 0.9	200 ± 67.8	38 ± 15.7

Table 4.2. Mean and range of DO saturation through the water column in cages with collector and a control, April-August 1999.

Cage	Mean DO (% saturation)	Range (% saturation)
NY	92 ± 5.3	87-97
WS	91 ± 4.3	86-95
Control	91 ± 3.7	84-97

The theoretical waste output for both cages was calculated from a waste mass balance approach using feed input data obtained from the fish farm and feed and biomass sample analyses taken throughout the monitoring period (Table 4.3). Material incorporated into fish biomass was accounted for by subtraction from the total feed input (Table 4.4). This value was then considered as the estimated waste load. Waste material was expressed in terms of SS and the composition as C, N and P was determined. It should be noted that these values represent maximum values for waste production. The effects of metabolism and respiration on the excretion of C, N and P as dissolved matter were not considered. The effects of the above mentioned processes were omitted in order to establish a "worst case" scenario for the efficiency of the collection system. All waste material was therefore assumed to be associated with the solid phase.

Table 4.3. Monthly estimates (wet weight) of biomass growth, fish size and number and total feed fed to the cages from fish farm production reports, April – August, 1999.

Date	Cage	Fish Weight (g)	Fish Number	Total Fish Biomass (kg)	Total Feed Fed Month^{-1} (kg)
07.04.99	WS	116.8	36,364	4,248	---
	NY	113.0	36,308	4,104	---
04.05.99	WS	126.2	35,733	4,510	604
	NY	129.7	35,866	4,652	643
01.06.99	WS	144.4	35,427	5,116	917
	NY	147.1	35,682	5,248	943
09.07.99	WS	205.0	34,250	7,011	1,397
	NY	208.0	34,579	7,194	2,180
17.08.99	WS	319.0	33,080	10,565	4,138
	NY	299.0	33,706	10,080	4,178

Table 4.4. Calculation of theoretical waste loading for WS and N cage and estimated of waste composition for the period April – August, 1999.

Parameter	Cage	SS	C	N	P
Dry weight of feed (kg)	WS	6,605	3,484	501	100
	NY	7,412	3,743	526	104
Dry weight gain of fish (kg)	WS	1,828	948	47	8
	NY	1,786	1,071	54	9
Feed-biomass = waste (kg)	WS	4,777	2,536	454	92
	NY	5,626	2,672	472	95
Waste retrieved from collector (kg)	WS	14.70	0.60	5.80	0.50
	NY	8.02	0.29	3.47	0.26
Efficiency of collector (%)	WS	0.31	0.02	1.27	0.53
	NY	0.14	0.01	0.73	0.27

It can be seen that whilst the level of waste outputs from the two cages used in the study differed (Table 4.4) in response to final biomass, feeding and growth (Table 4.3), this had little influence upon the mass of material recovered from the collector systems (Table 4.4). The greater mass of SS generated by cage NY may be attributed to the higher feed fed to this cage. Calculation of total feed fed to each cage (Table 4.3) indicates an estimated 888 kg (sum of monthly estimates) more feed was fed to cage NY. This results in the greater output of waste SS (849 kg) from the same cage. Also, the feed conversion ratios (FCR) were 1:1.12 and 1:1.33 for the WS and NY cage respectively, indicating a larger gain in biomass within cage WS. It may be expected that the quantities of C, N, and P as waste loading from cage WS would, as a result, be lower than in cage NY. However, there was a greater output of C, N, and P due to higher concentrations in feed samples from the WS cage and thus, the C, N and P waste loading was similar for both cages. The average dry content of feed analysed was 51.7 ± 2.1 % C, 7.3 ± 0.4 % N and 15.0 ± 2.2 mg P g^{-1} feed for the NY cage and 53.5 ± 1.1 % C, 7.7 ± 0.1 % N and 16.4 ± 1.9 mg P g^{-1}

feed for the WS cage. Collection rates, expressed as a fraction of the total waste budget, were extremely low (Table 4.4). The collection efficiency of both collectors was found to be less than 1 % SS through the study period.

4.3. PARTICLE SIZE ANALYSIS

Solid waste from *O. mykiss* which passed through the 2,000, 1,000 and 500 μm meshes was dominated by the <500μm fraction, which represented 34.4% of the total dry mass of sample (Table 4.5). Particle size analysis performed on the <500μm fraction revealed that the majority (90%) of particles had diameters of <493 and <544 μm for *O. mykiss* and *S. trutta* respectively. The estimated median diameters of this waste fraction were 278.8 ± 31.5 μm for *O. mykiss* and 304.4 ± 45.9 μm for *S. trutta* (Figure 4.2).

Table 4.5. The proportion (mean ± SD, n = 3) of particle size distribution of waste derived from experimental fish tanks.

Species	**Proportion (% dry weight)**			
	<500 μm	**500 – 1,000 μm**	**1,000 - 2,000 μm**	**>2,000 μm**
O. mykiss	34.4 ± 6.0	26.0 ± 9.3	22.3 ± 2.0	17.3 ± 3.2
S. trutta	23.2 ± 5.4	28.9 ± 5.1	25.1 ± 3.5	22.7 ± 6.0

Particle size analysis of the <500 μm fraction of collected wastes retrieved from WS and NY indicated that the median diameter of this fraction was 230.6 ± 38.6 μm and 215.7 ± 45.1 μm respectively where fish in both cages had MIW of 0.125 and 0.13 kg (WS and NY respectively) at the time of sampling. These values compare closely to the median particle diameter of the waste material from experimental tanks (Figure 4.2), indicating that the material entering the collector was similar in size range to that of faecal matter found in experimental investigations.

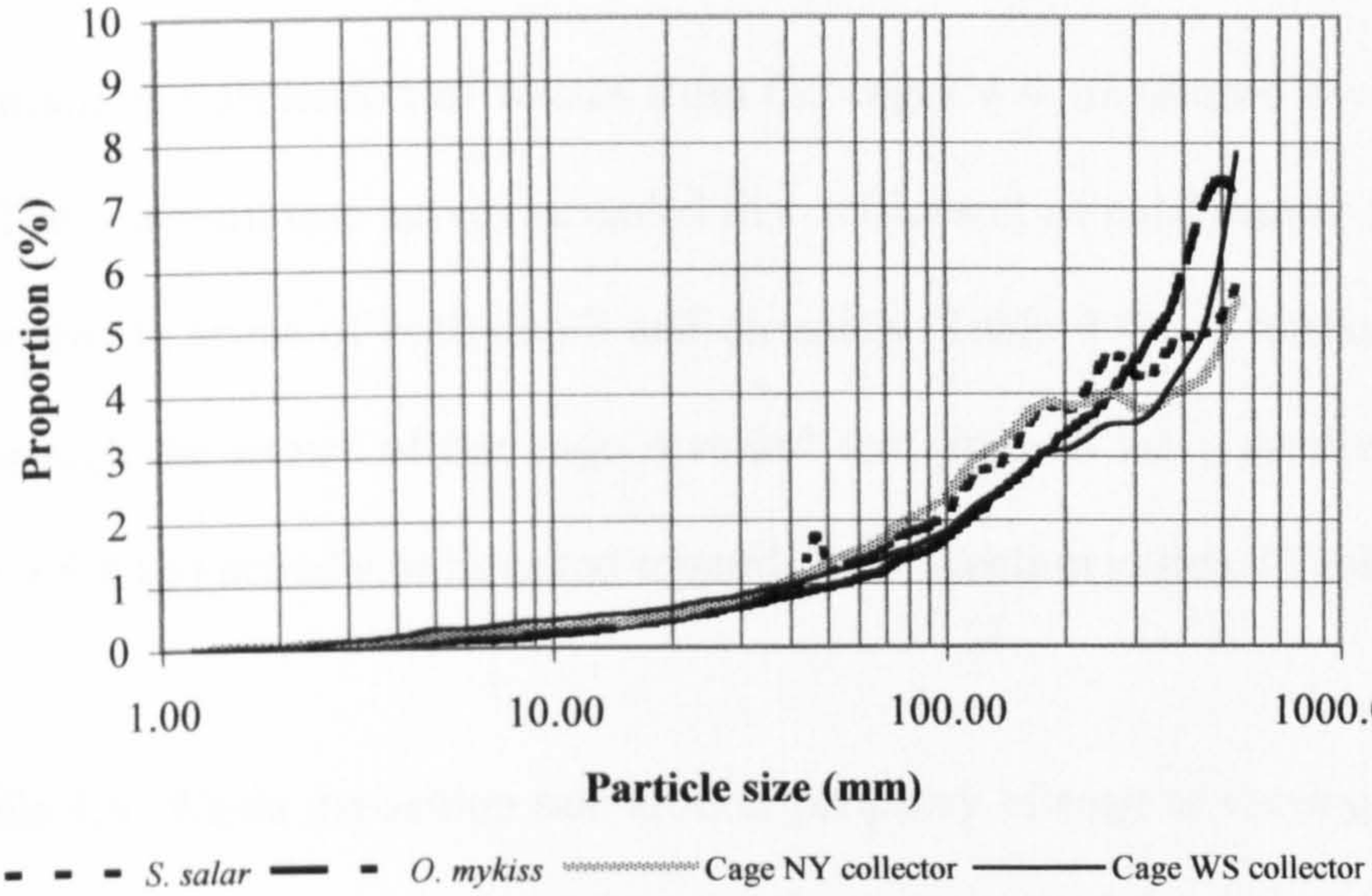


Figure 4.2. Particle size distribution of the <500 μm waste fraction from the experimental tanks and waste collection systems.

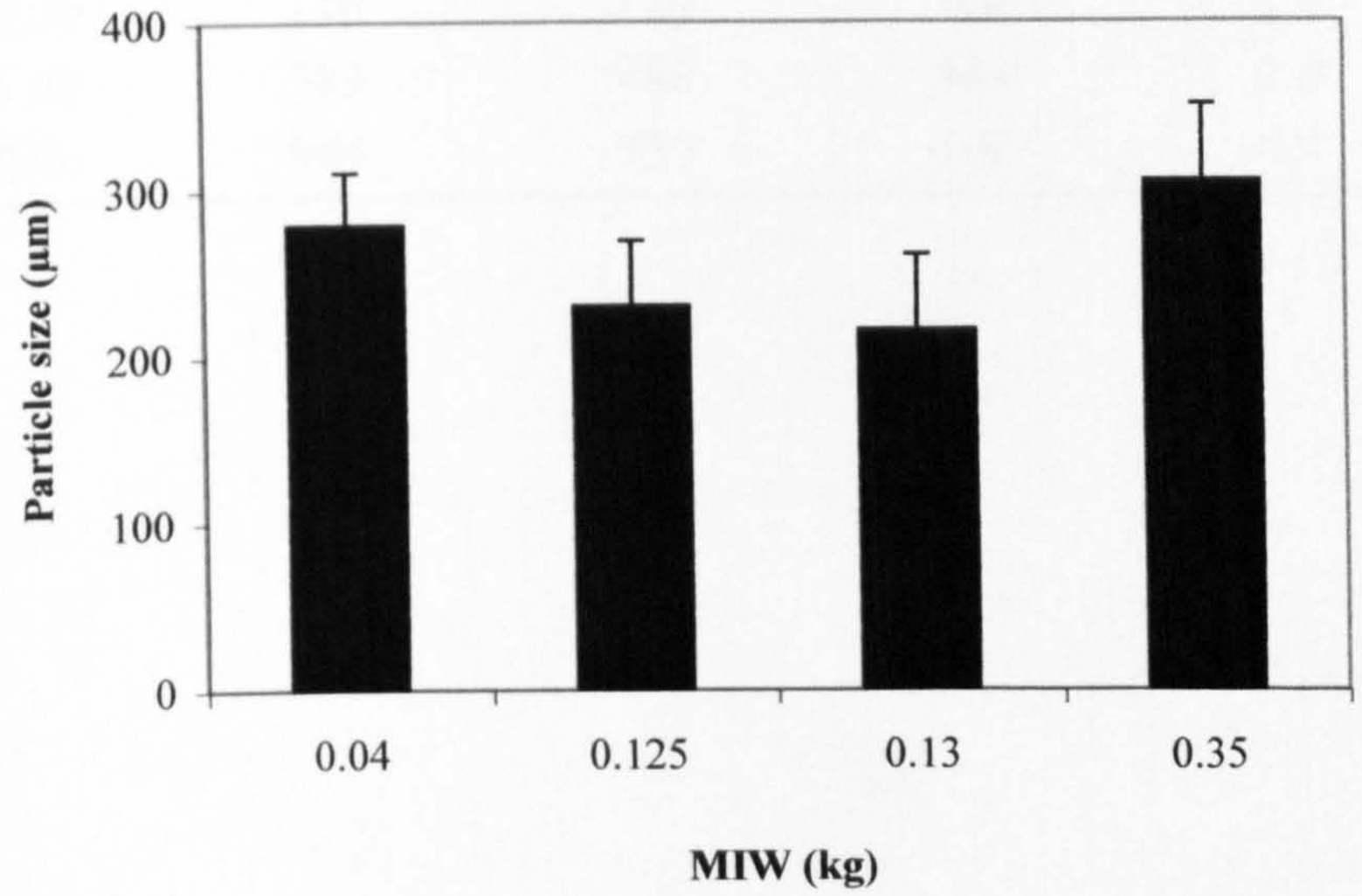


Figure 4.3. Median particle size diameters of waste material derived from experimental tanks and subcage collection systems containing fish with varying mean individual weights (MIW) (Mean ± SD, $n = 9$).

4.4. SEDIMENT TRAP SURVEY AND DAILY MONITORING

Sedimentation and dispersion of wastes from the cages was monitored over different time periods. The sediment trap survey revealed that settlement of solid matter in the cage was highly variable in terms of both depth and direction (Table 4.6). A further sediment trap survey through the centre of the cage revealed that little of the typical estimated waste load (not > 5.4 kg) actually sedimented towards the collection system (Table 4.7).

Table 4.6. Waste deposition rate around periphery of cage at varying depths and the corresponding mean and peak windspeeds for the duration of sediment trap deployment.

Date of survey	**Depth of trap (m)**			**Mean windspeed**	**Peak windspeed**
	0	**3.5**	**7.5**		
	Deposition rate (g m^{-2} day^{-1})			**(m s^{-1})**	**(m s^{-1})**
19.06.99	183	172	185	2.6	4.1
10.07.99	130	140	106	1.9	4.9
17.05.00	389	486	444	2.0	3.4
24.05.00	944	750	792	0.9	2.5

Table 4.7. Waste deposition rate (Mean ± SD, n = 3) through the centre of the cage and the corresponding mean and peak windspeeds for the duration of the sediment trap deployment.

Date of survey	Depth of trap (m)			Mean windspeed	Peak windspeed
	0	3.5	7.5		
	Deposition rate (g m^{-2} day^{-1})			($m\ s^{-1}$)	($m\ s^{-1}$)
28.10.99*	36 ± 0.2	30 ± 0.1	36 ± 1	2.1	5.7
18.05.00	167 ± 0.1	150 ± 5	140 ± 3	3.2	5.6
24.05.00	347 ± 93	250 ± 31	150 ± 7	1.6	4.2

*Note: Sediment trap survey carried out over 7 day period and normalised for daily capture.

Removal of waste solids from the collection device was undertaken on a weekly basis. Therefore, with a view to enhancing collection efficiency, daily retrieval of waste from the collection systems was monitored on six occasions over different periods (Table 4.8).

Table 4.8. Estimated solid waste load to cage NY and total waste retrieved via collection system during daily monitoring of waste collector performance.

Date	Waste	SS (kg)	P (kg)	C (kg)	N (kg)
19.06.99	Load	78.900	2.00	39.600	7.500
	Collected	0.156	0.006	0.056	0.005
	% Efficiency	0.197	0.300	0.141	0.066
20.06.99	Load	71.500	1.600	24.500	6.700
	Collected	0.079	0.003	0.028	0.002
	% Efficiency	0.110	0.188	0.114	0.029
21.06.99	Load	697.700	17.500	507.300	77.600
	Collected	0.090	0.004	0.032	0.002
	% Efficiency	0.013	0.023	0.006	0.003
10.07.99	Load	122.200	2.400	71.800	12.200
	Collected	0.289	0.011	0.099	0.008
	% Efficiency	0.24	0.458	0.138	0.065
11.07.99	Load	123.500	1.100	86.000	4.300
	Collected	0.589	0.022	0.197	0.018
	% Efficiency	0.477	2.000	0.229	0.419
12.07.99	Load	926.200	20.500	734.100	105.600
	Collected	0.308	0.011	0.108	0.008
	% Efficiency	0.033	0.054	0.015	0.008

4.5. METEOROLOGICAL DATA AND WATER VELOCITIES

Meteorological data, specifically wind run, air and surface water temperature were monitored during the trial period. Average wind speed for the period was approximately 2.3 m s^{-1} with a maximum velocity of 8.6 m s^{-1} (Table 4.9). Water and air temperatures peaked as expected during summer months with concurrent values of 20.4 and 27.3°C respectively.

Table 4.9. Meteorological data recorded during the waste collection trial period, April-August, 1999.

Month	Air Temperature (°C)		Water Temperature (°C)		Windspeed ($m\ s^{-1}$)	
	Min	Max	Min	Max	Min	Max
April	-1.6	20.6	4.9	8.8	0.02	8.6
May	3.6	20.2	5.9	11.1	0.03	7.9
June	5.1	22.7	8.8	15.3	0.02	6.2
July	6.6	27.3	12.2	20.4	0.00	6.4
August	4.3	22.6	15.3	18.3	0.07	5.4

Measurement of water current velocities (*Section 3.2.6*) undertaken at the site indicated that all three methods of measurement were comparable (Table 4.10). Using the range of estimated mean and peak water current velocities at depths between 1 – 4 m, it was possible to correlate these values with the recorded windrun during the period of water velocity measurement. Comparison, therefore, of water current velocity as a percentage of wind speed during the deployment of the current measurement instrumentation gave an average value of *c.* 1.4%.

Using this percentage it was possible to estimate the current speeds generated by wind during the period of subcage collection system deployment. The mean current velocity for the deployment of the subcage collection system (April – August, 1999) was 0.031 m s^{-1} with a maximum value of 0.120 m s^{-1} (Figure 4.4).

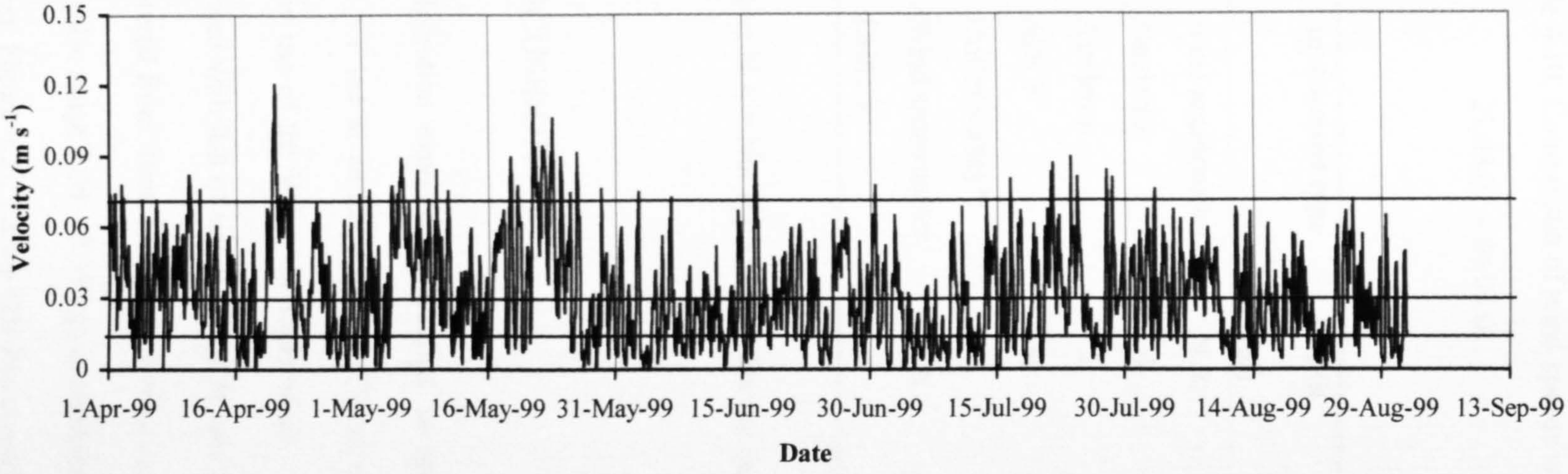


Figure 4.4. Water current velocities estimated from wind speeds during the period of subcage collection system deployment April-August, 1999. Horizontal gridlines represent estimated fall velocities for faecal particles (0.015 – 0.030 m s^{-1}) and feed pellets (0.07 m s^{-1}).

Table 4.10. Comparison of wind speeds with methods of current velocity measurement, 21.03.01 – 04.04.01.

Instrument type	Depth (m)	Mean speed ($m\ s^{-1}$)	Peak speed ($m\ s^{-1}$)
Wind anemometer	N A	2.71	8.67
Aanderaa	4	0.03	0.14
Aanderaa	1	0.03	0.13
ADCP	4	0.03	0.09
Drifter survey*:			
Wind anemometer	N A	6.22	7.60
Drifters	3	0.11	0.15

Note: N A = Not applicable; * Survey carried out on 21.03.01.

4.6. DISCUSSION

Net configuration experiments resulted in approximately only 30% collection efficiency using the NY net at an angle of 45° (Figure 4.1). The efficiency of collection was further reduced by use of the WS at an angle of 20 – 30° (*c.* 23%). Cages with collection systems were stocked with fish of size 0.11 kg. It may therefore be assumed that the particle size of faecal material from these fish would have a range similar to that of faecal material obtained from fish (size range 0.04 – 0.35 kg) in experimental tanks (median diameter range 278.8 μm - 304.4 μm, Figure 4.2). Taking this into consideration and also the <500 μm fraction having the greatest percentage proportion of the total waste output (Table 4.5), the potential collection efficiency of the WS net may appear low. However, a number of studies have

shown that an increase in waste load enhances screening effectiveness (Cripps, 1995; Kelly et al., 1997) and sedimentation of wastes (Bergheim et al., 1998). Cripps (1995) argued that the screening process improved with greater waste loads due to the build up of a filter cake that would restrict the passage of particles smaller than the nominal pore diameter. Bergheim et al. (1998) found that the sedimentation of aquaculture effluents increased from 58% at 1 mg SS min^{-1} to 90% at 18 mg SS min^{-1}. This was probably due to the agglomeration of particles in more concentrated suspensions, thus increasing their mass and thereby settling faster. This was also observed in this study during net configuration experiments. The process of coalescence would also serve to enhance the screening process and hence, the collection efficiency.

The presence of the waste collection device did not affect the water quality within the cage. Dissolved oxygen levels were more than comparable to that of the control cage (> 90% saturation). Similarly, levels of TP and TAN were also close to those of the control cage indicating no adverse affect on water quality due to the presence of the collection system.

Suspended solids removal from both cages was extremely poor. Behmer et al. (1993) reported a 100% increase in the recovery of solids when the rate of waste removal from the collection device was increased from 72 h periods to a daily basis. However, efficiency of collection in this study was not enhanced significantly with increased emptying of the waste collector (Table 4.8). It should be noted however that the experimental cage and collection system Behmer et al. (1993) employed was only *c.* 1 m deep. Cages in this study had a depth of 9 m, greatly increasing the potential for waste dispersion before capture by the collection device.

The theoretical waste load estimated during this study was a "worst case" scenario for the performance of the collection system. Previous studies have shown (*Section 1.3.2.1,* Table 1.6) that the dissolved fraction may account for a significant proportion of waste lost to the environment. Similarly, the effects of leaching and dissolution on waste material, which would increase the soluble waste fraction, were not taken into account. Elberizon (2000) estimated that the degree of leaching of C and N from faeces at a fall velocity of 0.03 m s^{-1} and depth of 9 m (also the depth of collection system) was 3.7 and 15.1% respectively. In the same study, over a period of 15 days at a temperature of 10°C, dissolution of C and N was estimated at 12.1 and 48.0% respectively. Similarly, leaching and dissolution of P has been investigated in freshwater (Behmer et al., 1993; Kelly, 1993; Garcia-Ruiz and Hall, 1996) with estimates ranging from 11.1 – 64% for leaching of P after 24 h. Kelly (1993) estimated the release of P from sediments underneath cages at a rate of 1.5 – 57.6 mg P m^{-2} d^{-1}, with variation in release rates associated with the intensity of cage farming on the sampled sites.

The effects of leaching and dissolution on the waste alone cannot explain the poor collection efficiency. For example, a carbon mass balance of the cage system on daily waste retrieval of collected wastes reveals that there was a mean theoretical waste loading of 244 kg C (Table 4.8). If the maximum estimated values for losses of C, because of the dissolved fraction (69%, including respiratory and urinary C) and the processes of leaching (3.7%) and dissolution (12.1%), are considered, 64 kg C could potentially be retained in the collection system. However, the mean value of retrieved waste during the daily monitoring of the system was only 0.09 kg C (Table 4.8).

Sediment trap surveys revealed it was the tendency for loss of material through the sides, rather than the bottom of the cage, which severely limited the capacity of the collector to

intercept and adequately trap the wastes produced during production (Table 4.6). This hypothesis is confirmed by sediment trap surveys through the centre of the cage which revealed that a mean of approximately 0.07 kg waste kg^{-1} feed consumed deposited to the bottom of the cage (Table 4.7). However, using mean values for sediment trap waste recovery from the periphery of the cage, *c.* only 30% of the total waste output for the cage was accounted for. The position of the sediment traps around the cage and the single point mooring system of the cages may partly explain the deficit in waste budget. The movement of the cage into the wind around the single point mooring creates localised dispersion towards the leeward part of the cage. Sediment traps were positioned at the centre of each side thus allowing for the possible dispersion of waste through the corner of the cage without being recorded.

The loss of waste through the sides of the cage may be attributed to water currents in the lake. Elberizon and Kelly (1998) estimated that the fall velocities of faecal particles in freshwater ranged from 0.015 – 0.030 m s^{-1}. Therefore, for realistic collection efficiencies, water currents should be less than the fall velocities of the faecal particles. In the same study, the median fall velocity for a variety of feed pellets typically used in freshwater salmonid production was estimated at 0.07 m s^{-1}.

Wind is the primary force causing water motion in lakes at all depths. (Smith, 1992; Horne and Goldman, 1994). Wind energy at the water surface is transferred to the water column generating horizontal advection flow and turbulence in the upper layers. Turbulent mixing may be defined as the random, three dimensional motion of small volumes of water within the water column and is parameterised as the eddy diffusivity (Smith 1992; Chapra, 1997).

The downward transport of kinetic energy creates drift currents at lower levels. These are the basic processes creating wind driven motion in lakes. (Webster, 1990; Smith, 1992).

Estimation of the water current velocities during the deployment of the undercage collection device revealed the mean velocity to be 0.031 m s^{-1}. Using a simple dispersion model, the potential for the deposition of waste to the collection device may be estimated. Gowen et al. (1989) designed a predictive waste dispersion model to assist in the evaluation of sites for cage aquaculture. The horizontal displacement of waste material from the cage may be estimated using the following equation:

$$d = \frac{D \times C_V}{V}$$

where d is the distance dispersed; D is the depth of sedimentation; C_V is the water current velocity and V is the fall velocity of the waste material, faecal matter or waste feed respectively (Midlen and Redding, 1998). Using a water depth of 10 m for the collection device, the estimated mean water current velocity and faecal matter fall velocity of 0.030 m s^{-1}, it can be predicted that waste material was dispersed *c.* 10 m from the subcage collection system. Assuming the same current velocity, it may also be predicted that waste feed had a horizontal displacement of about 4.5 m.

This demonstrates that there exists more than sufficient water velocity for almost 45% of the monitoring period (April – August, 1999) at this site to deflect waste particles away from the cages and hence contribute to the observed poor capture rate of the collection device. If the lower fall velocity of 0.015 m s^{-1} is considered then the percentage of current velocities

capable of deflecting waste material from the cage during the deployment period increases to almost 70%.

The influence of wind energy on the displacement of waste from the cages was further demonstrated by the determination of horizontal dispersion coefficients at the study site. Dispersion in the *X* direction (West) was approximately three times greater during the survey period, due to higher wind speeds (Table 4.11). Similarly, k_y dispersion coefficients were an order of magnitude higher (Table 4.11). These results demonstrate considerable potential for horizontal displacement of solid waste in the water column at the study site.

Table 4.11. Range of dispersion coefficients determined at Loch Earn over two survey periods (Cromey and Provost, 2000; 2001).

Survey	k_x ($m^2 s^{-1}$)	k_y ($m^2 s^{-1}$)	Mean windspeed ($m s^{-1}$)	Peak windspeed ($m s^{-1}$)
21.11.00	0.002 – 0.024	0.001 – 0.014	2.45	2.77
21.03.01	0.001 – 0.070	0.000 – 0.169	6.22	7.60

Visual examination of the recovered sediment traps revealed that the proportion of uneaten or partially eaten feed pellets in the collected material increased with trap depth. This observation would be consistent with the active dispersion of material due to water currents, allowing only heavier material to sediment towards the collector. In comparison with faecal waste material, particle size analysis of wastes retrieved via the collection system was similar in size indicating that the majority of waste retrieved was actually faecal material. This may

be explained by the break up of feed pellets due to physical disintegration caused by the airlift system and also natural processes such as dissolution.

Further factors to be considered regarding sub cage collection should include the contribution of the fish themselves within the cage to waste dispersal. Activity was, as expected, observed to be greatest during feeding. Fish induced currents during this period would greatly increase the water current velocity within the upper part of the cage thus potentially enhancing the dispersion of waste feed and faecal material.

Although the evaluation of such undercage collection devices has been previously studied, their feasibility for use in Scottish freshwater environments has not been attempted. More importantly, published data relating to the physical measurement of water current velocities in the freshwater lake environment (both Scottish and globally) could not be found by the author. Such data is not known to readily exist due to the difficulties associated with measurement of water movements in lakes, primarily because of the sophisticated equipment required to do so. It has only been in the last two decades that such equipment has been commercially developed and readily available. In light of this, data provided in this research concerning lake water current velocities and dispersion co-efficients most be considered as valuable contributions to the fields of water hydrodynamics and Scottish limnology.

Chapter 5

The Anaerobic Digestion of Aquaculture Effluents

5.1. ANAEROBIC DIGESTION

The potential for the treatment of aquaculture effluents by means of anaerobic digestion was investigated using reactors as described in *Section 3.3.1*, initially under mesophilic temperatures at a HRT of 11.67 days and subsequently under mesophilic (35°C), thermophilic (55°C) and psychrophilic (ambient temperature, 15 - 22°C) temperatures at a HRT of 23.33 days. Biogas, feed and effluent samples were analysed as described in *Sections 3.3.3* and *3.3.4* respectively.

5.1.1. Experimental Procedure

The mesophilic digester was seeded with 3 l digested sewage sludge (TS: 39,960 mg l^{-1}) and 1 l aquaculture waste (TS: 21,280 mg l^{-1}) on day 0 of the study (*Section 3.3.2*), the resulting mixed liquor having a TS concentration of 35,290 mg l^{-1}. Feeding commenced on day 2, with 0.8 l settled digester liquor withdrawn and replaced with aquaculture waste 3 times per week to give an initial HRT of 11.67 days. No feed was added over the holiday period from day 68 until day 85, when the quantity of feed added 3 times per week was changed to 0.4 l to produce a HRT of 23.33 days. This HRT was maintained for the remainder of the experiment. When a part of the digester contents were removed to start up the thermophilic digester on day 100, as described below, the working volume was restored to 4 l by adding aquaculture waste without withdrawal of an equivalent volume of liquor for the next three feeds.

The thermophilic digester was seeded with 2 l digested sewage sludge (TS: 17,410 mg l^{-1}), 1 l sludge taken from the mesophilic reactor (TS: 51,920 mg l^{-1}) and 1 l aquaculture waste (TS: 11,220 mg l^{-1}) on day 100, giving an initial mixed digester liquor TS of 24,490 mg l^{-1}. To accomplish the transition to thermophilic operation, digester temperature was kept at 36°C for one week after seeding, increased to 55°C in 5°C increments over the next 12 days and maintained at this temperature for a further week. No feed was added during this transition period. From day 126 onwards, 0.4 l feed was added 3 times per week to give a HRT of 23.33 days.

The psychrophilic digester was seeded with 2 l digested sewage sludge (TS: 19,428 mg l^{-1}), 0.8 l aquaculture waste (TS: 15,130 mg l^{-1}) and 1.2 l distilled water on day 124, giving an initial mixed digester liquor TS of 24,490 mg l^{-1}. As with the other two digesters during this period of operation, 0.4 l feed was added 3 times per week to give an HRT of 23.33 days.

5.1.2. Anaerobic Digestion of Aquaculture Effluents

Mixed digester liquor TS and SS values are presented in Figure 5.1. The solids content of the mesophilic digester increased significantly from start-up until day 44. The subsequent reductions between days 50 and 85 were probably due, at least in part, to the lack of feed for the digester during much of this period. After day 85, with a HRT of 23.33 days, the mesophilic digester solids content was more stable at *c.* 45,000 mg l^{-1} for both TS and SS. The psychrophilic and thermophilic digesters both had fairly constant TS and SS contents after start-up at a HRT of 23.33 days.

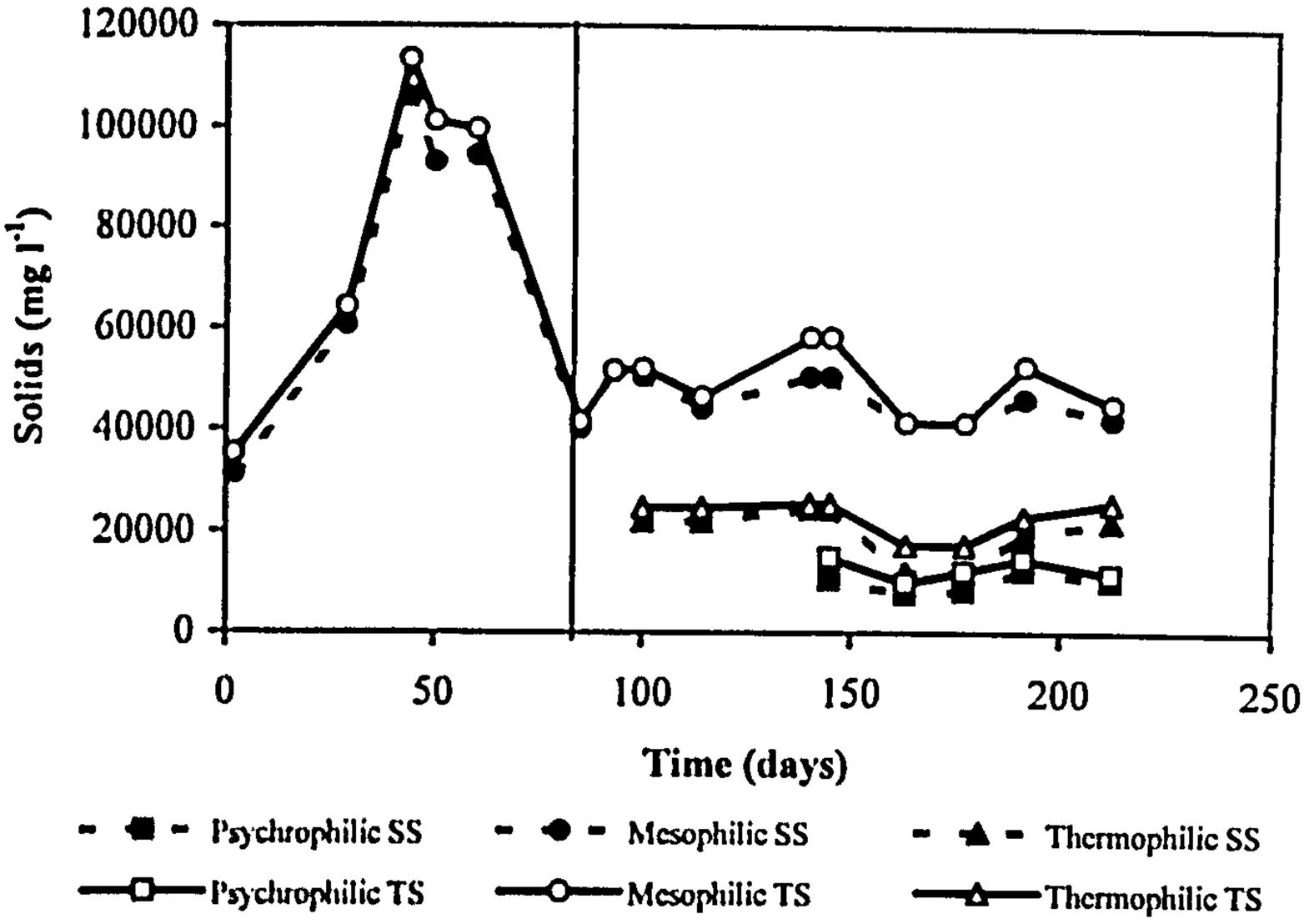


Figure 5.1. Digester mixed liquor TS and SS concentrations.

The performance of the mesophilic digester was variable during the initial part of the study prior to day 85, when HRT was 11.67 days (Table 5.1). Solids removal was reasonable, with similar trends observed for both TS and SS. Similarly, COD and BOD removal were also satisfactory, but some of the analytical results suggest that this should be considered an acclimation period. Biogas production was relatively low at *c.* 1 l day^{-1} or less but then increased after day 26 to over 3 l day^{-1} for around 3 weeks, after which it declined slowly to around 1.5 l day^{-1}. Meanwhile, total VFA fell from over 2,000 mg l^{-1} on day 23 to around 200 mg l^{-1} by day 63. These results, together with the accumulation and then loss of solids described above, imply that the rates of solids digestion and VFA conversion were initially poor, resulting in a solids build-up within the digester with low biogas production, but a later increase in biological activity allowed the digestion of these accumulated solids with a corresponding increase in biogas production. The initial slow rate of VFA conversion and hence poor biogas yield may be explained by the slow growth rate of methanogenic bacteria as discussed in *Section 2.6.*

Table 5.1. Summary of digester performance in terms of percent removal at varying temperature and hydraulic retention times. Mesophilic digester values in brackets (days 46 - 65) represent performance under stabilised conditions.

Parameter	HRT = 11.76 days	HRT = 23.33 days		
	Mesophilic	Psychrophilic	Mesophilic	Thermophilic
COD	26 (36)	72	76	60
BOD	78 (88)	79	97	89
TS	50 (70)	62	60	48
SS	66 (77)	81	70	57
P	56 (61)	76	83	55

TS, SS and COD data for the entire period of digestion for all three digesters are presented in Figures 5.2 - 5.4. There was considerable variation in the feed composition over time, which was difficult to avoid given the nature of the feed material and the aquaculture waste collection method employed (*Section 3.3.2.*). It is evident that all three digesters were effective in removing solids and COD. Effluent solids concentrations were consistently lower than the feed values, the only conspicuous exceptions being the high values recorded for the mesophilic digester effluent on days 42, 98 and 117. The most likely explanation for these high values is sampling error, possibly due to floating sludge being drawn into the effluent sampling tube at the top of the digester. Effluent COD concentrations were also generally lower than those of the feed. Statistical analysis (one-way ANOVA) of effluent concentrations for all three reactors, revealed that was no significant difference in TS (where $n = 21$, $F_{2, 18} = 2.06$, $P < 0.05$), SS (where $n = 18$, $F_{2, 15} = 3.14$, $P < 0.05$) and COD (where $n = 33$, $F_{2, 30} = 1.00$, $P < 0.05$).

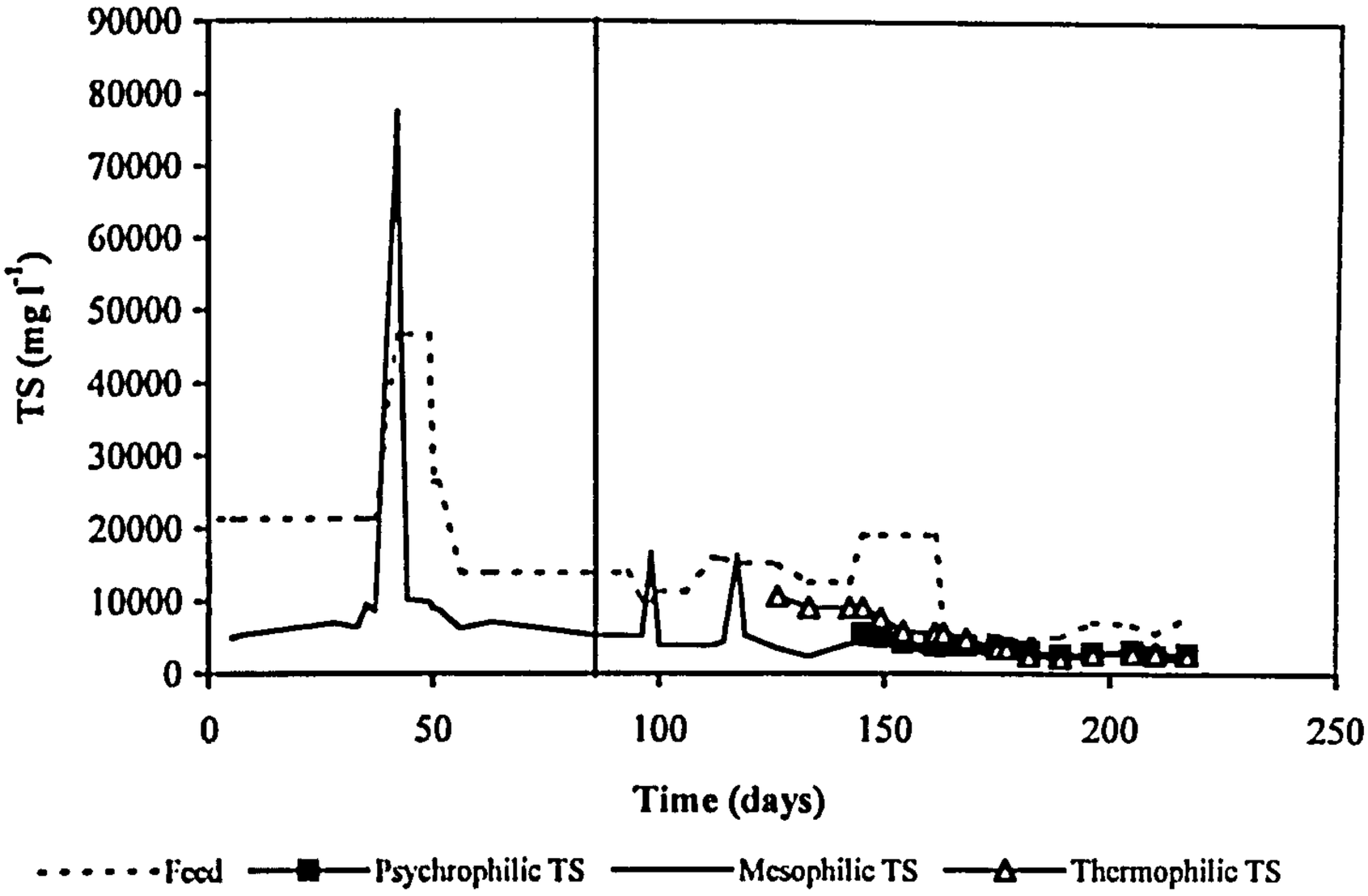


Figure 5.2. Feed and effluent TS concentration for all three reactors.

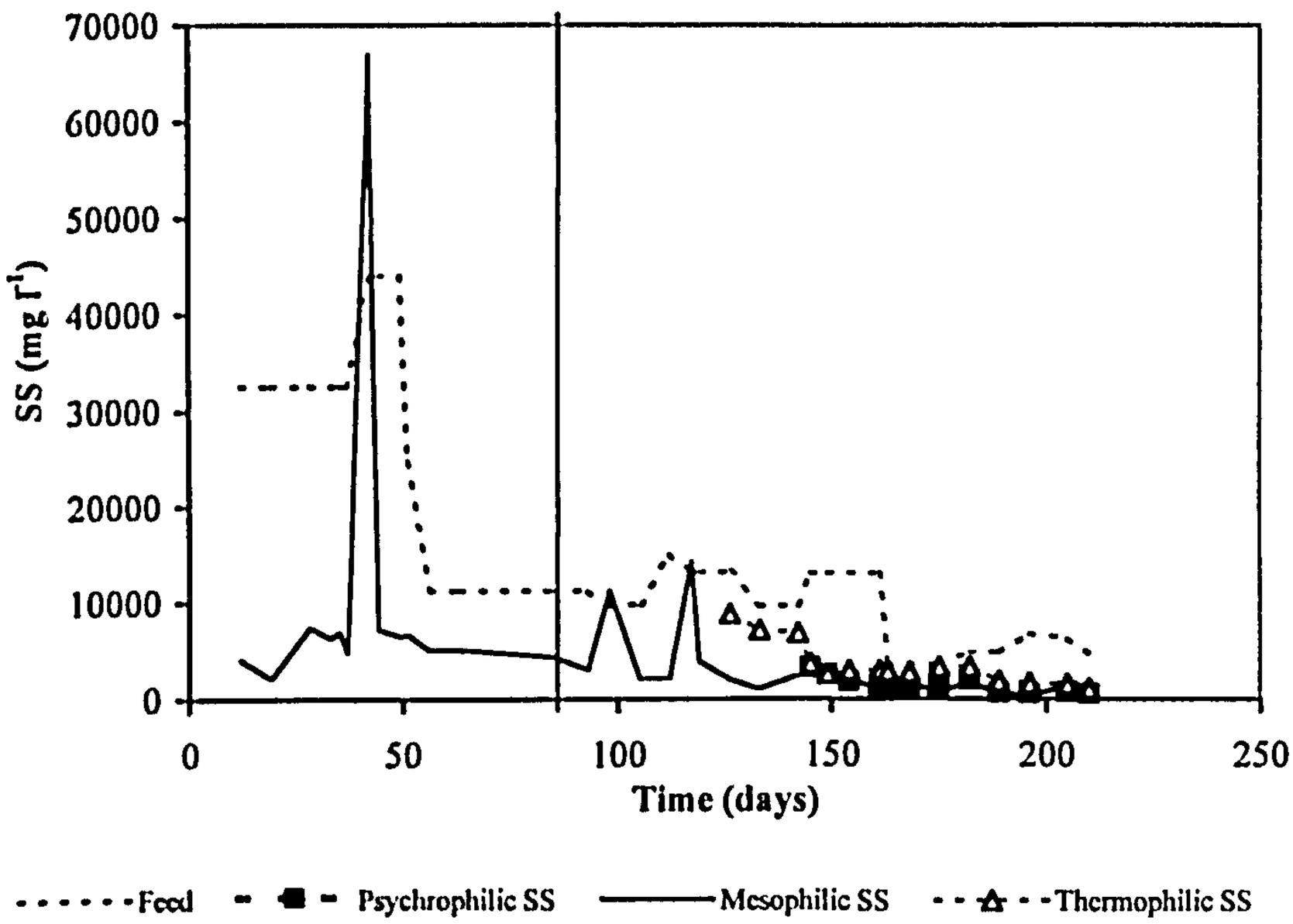


Figure 5.3. Feed and effluent SS concentrations for all three reactors.

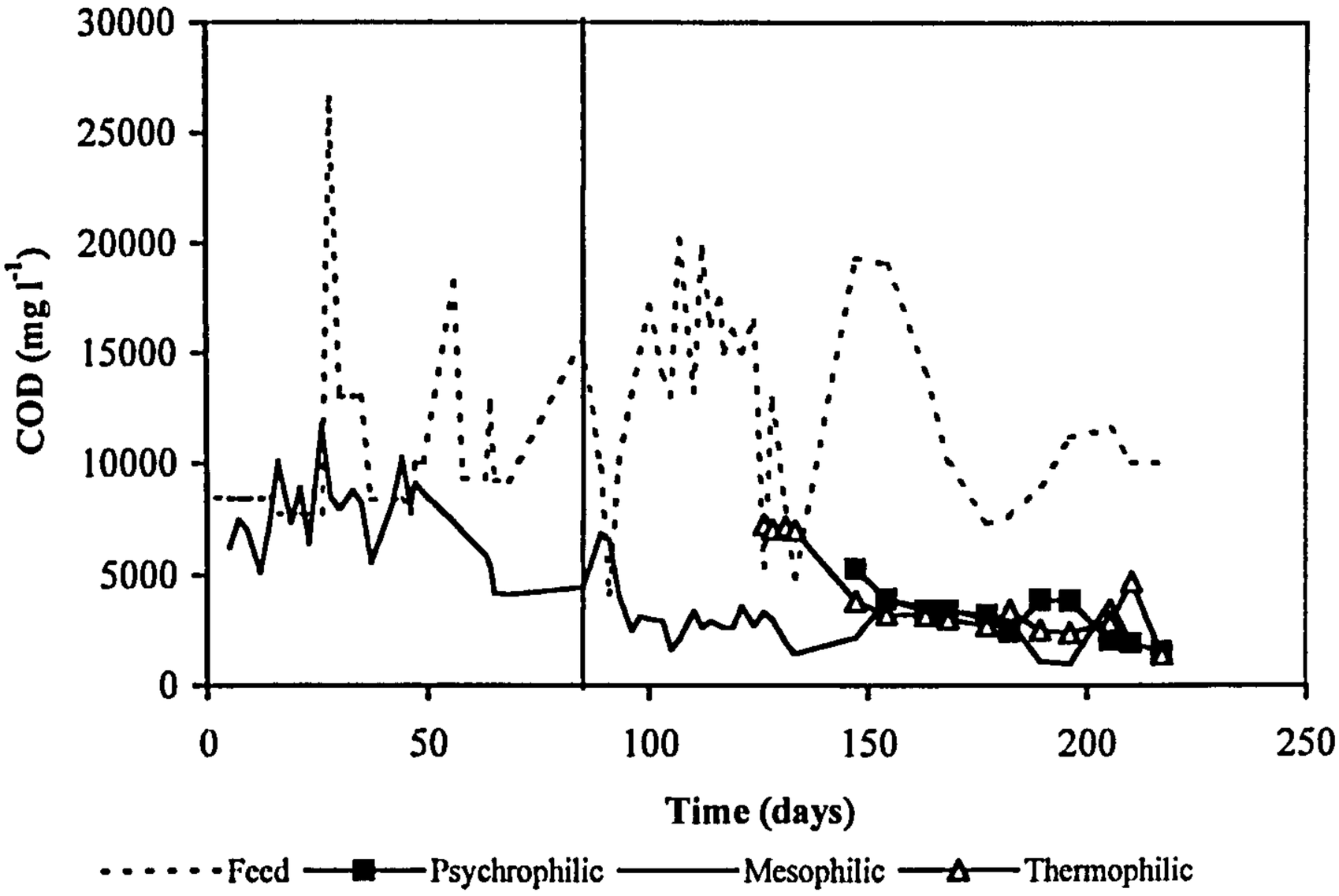


Figure 5.4. Feed and effluent COD concentrations for all three reactors.

Soluble COD values (Figure 5.5) were much lower than the unfiltered effluent COD values. The difference in mean soluble COD effluent concentrations at all three operating temperatures, where $n = 38$ in each case, was not statistically significant ($F_{2, 35} = 2.26$, $P < 0.05$). The mesophilic digester effluent had a soluble COD around 2,500 mg l^{-1} on day 21, which fell to 336 mg l^{-1} by day 63 and remained in the range 150 - 700 mg l^{-1} thereafter. The soluble COD of the psychrophilic digester effluent remained below 700 mg l^{-1} throughout the study. The thermophilic digester effluent soluble COD was 3,464 mg l^{-1} on day 126, immediately after the transition from mesophilic operation, but fell consistently after this, falling to 342 mg l^{-1} by day 147 and remaining below 600 mg l^{-1} for the remaining part of the study. It has been demonstrated that increasing the temperature from 35°C to 55°C results in the decay of a large proportion of the bacteria initially present (Van Lier et al., 1990). It may therefore be reasoned that soluble cell constituents were released into the medium as a result of bacterial death, contributing to the large increase in soluble COD. These soluble compounds would then have been either

metabolised by other bacterial species, broken down by enzyme action or washed out of the digester over time. The observed fall in soluble COD levels is much greater than that which would be predicted from washout alone within days 126 - 147.

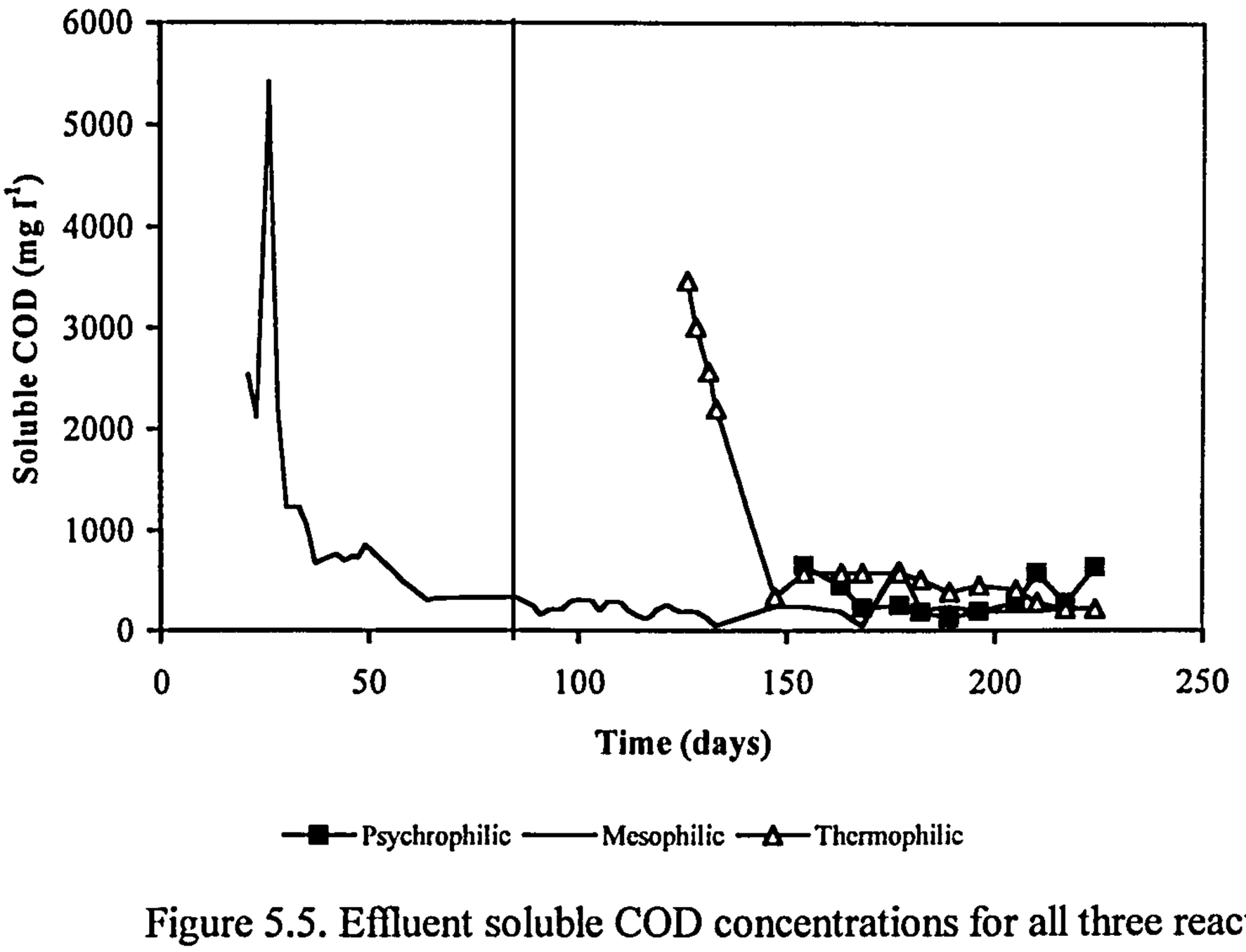


Figure 5.5. Effluent soluble COD concentrations for all three reactors.

Mesophilic digester effluent BOD values ranged from 500 - 1,000 mg l^{-1} during the initial period of the experiment with HRT 11.67 days; after this, with HRT 23.33 days, they were consistently below 300 mg l^{-1} and typically around 150 mg l^{-1}. Thermophilic digester effluent BOD values declined from 1,200 mg l^{-1} on day 133 to around 300 mg l^{-1} by the end of the study, while psychrophilic digester effluent values were higher at around 1,000 mg l^{-1} from day 175 onwards. The feed BOD/COD ratio was somewhat variable but typically around 0.3, indicating a moderately biodegradable effluent, but the mesophilic digester effluent BOD/COD ratio was consistently around 0.08, suggesting that much of the residual organic material left after treatment was recalcitrant in nature. Effluent BOD/COD ratios for the thermophilic digester were slightly higher than those

for the mesophilic system at around 0.13, but psychrophilic digester effluent BOD/COD ratios were closer to those of the feed.

In terms of cumulative biogas production rates, the mesophilic digester produced more biogas in comparison with the other reactors at HRT 23.33 days (Figure 5.6). The mesophilic reactor produced more biogas (mean = 1.76 ± 0.6 l day^{-1}) than the thermophilic (mean = 0.4 ± 0.25 l day^{-1}) digester between days 100 and 140, but biogas outputs from the two digesters were very similar after this (0.50 ± 0.15 and 0.52 ± 0.2 l day^{-1} for the mesophilic and thermophilic digesters respectively) although still statistically significantly different (where $n = 140$, $F_{1, 138} = 0.513$, $P < 0.05$). This pattern would be expected because the thermophilic digester was in a transitional phase from day 100 to 126, with no feed added, but would have developed a stable thermophilic population after this. Biogas production from the psychrophilic digester was lower than that from the other two digesters. Analysis of the biogas composition from all three digesters between days 142 and 212 indicated that biogas CH_4 content was reasonably constant at around 60%.

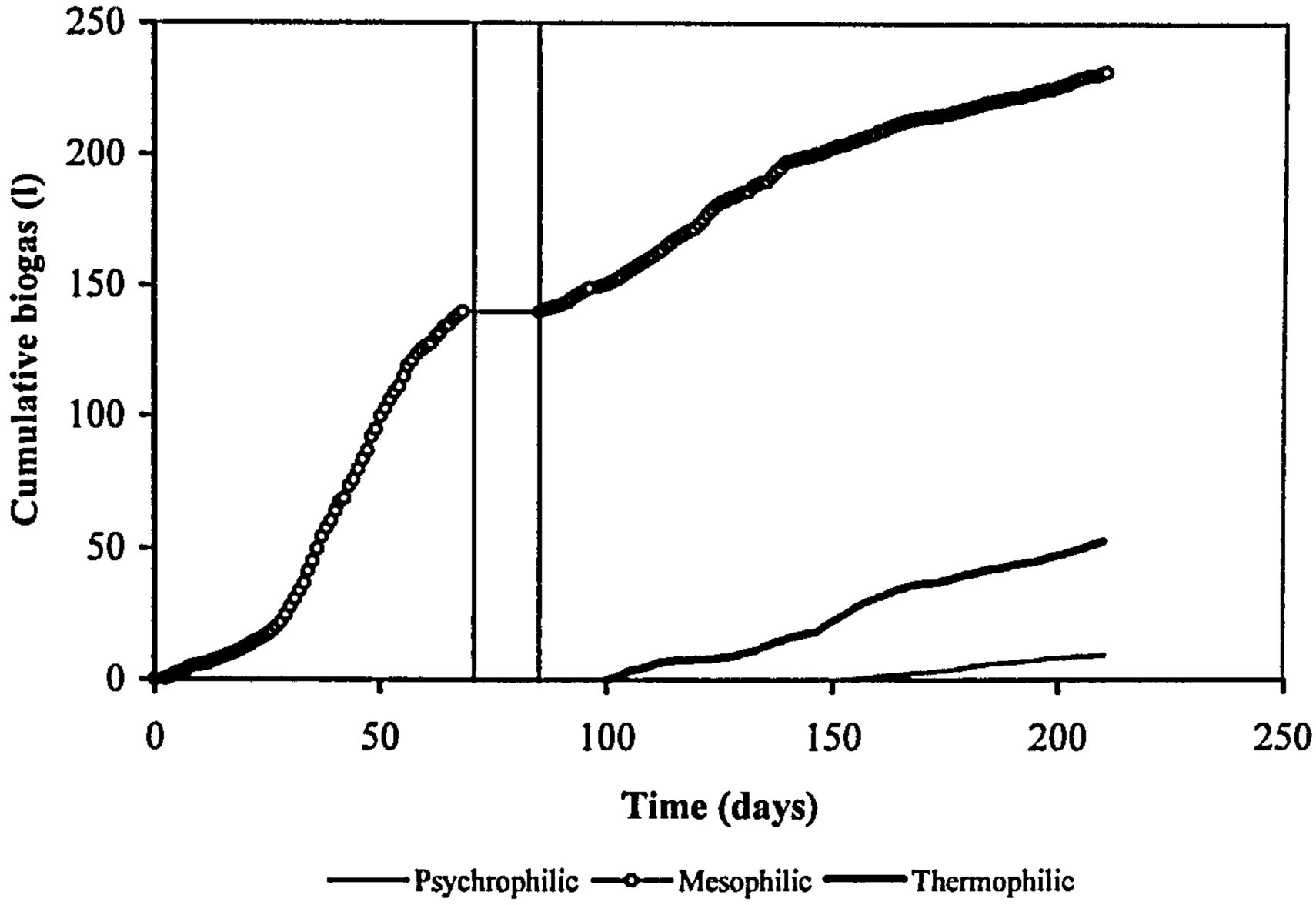


Figure 5.6. Cumulative biogas production for all three reactors for the duration of the experiment.

Mesophilic digester pH was 6.2 on day 2, but this rose rapidly to over 6.9 by day 28 and remained in the range 6.9 - 7.2 thereafter. Thermophilic digester pH rose from 7.2 on day 100 to 8.1 on day 137, then fell to 7.1 by the end of the study. The high pH around day 137 reflected the high level of feed Alk used during start-up to ensure the transition to thermophilic operation proceeded without digester failure. The psychrophilic digester had an initial pH of 7.3, which fell to 6.4 by day 131 then stabilised in the range 6.8 – 7.0 for the rest of the study. Digester alkalinities were maintained at *c.* 3,000 mg $CaCO_3$ l^{-1}, although there were peaks of up to 8,000 mg $CaCO_3$ l^{-1} after additional sodium hydrogen carbonate had been used for pH adjustment. VFA concentrations (Figure 5.7) were generally in the range 300 - 2,500 mg l^{-1} in total, the peaks occurring during periods of stress (*e.g.* in the thermophilic digester during start-up and between days 145 and 161 when feed TS, SS and COD were particularly high). VFA levels tended to be higher in

the psychrophilic digester than in the other two, and propionic acid levels tended to be higher than acetic acid levels in all three cases.

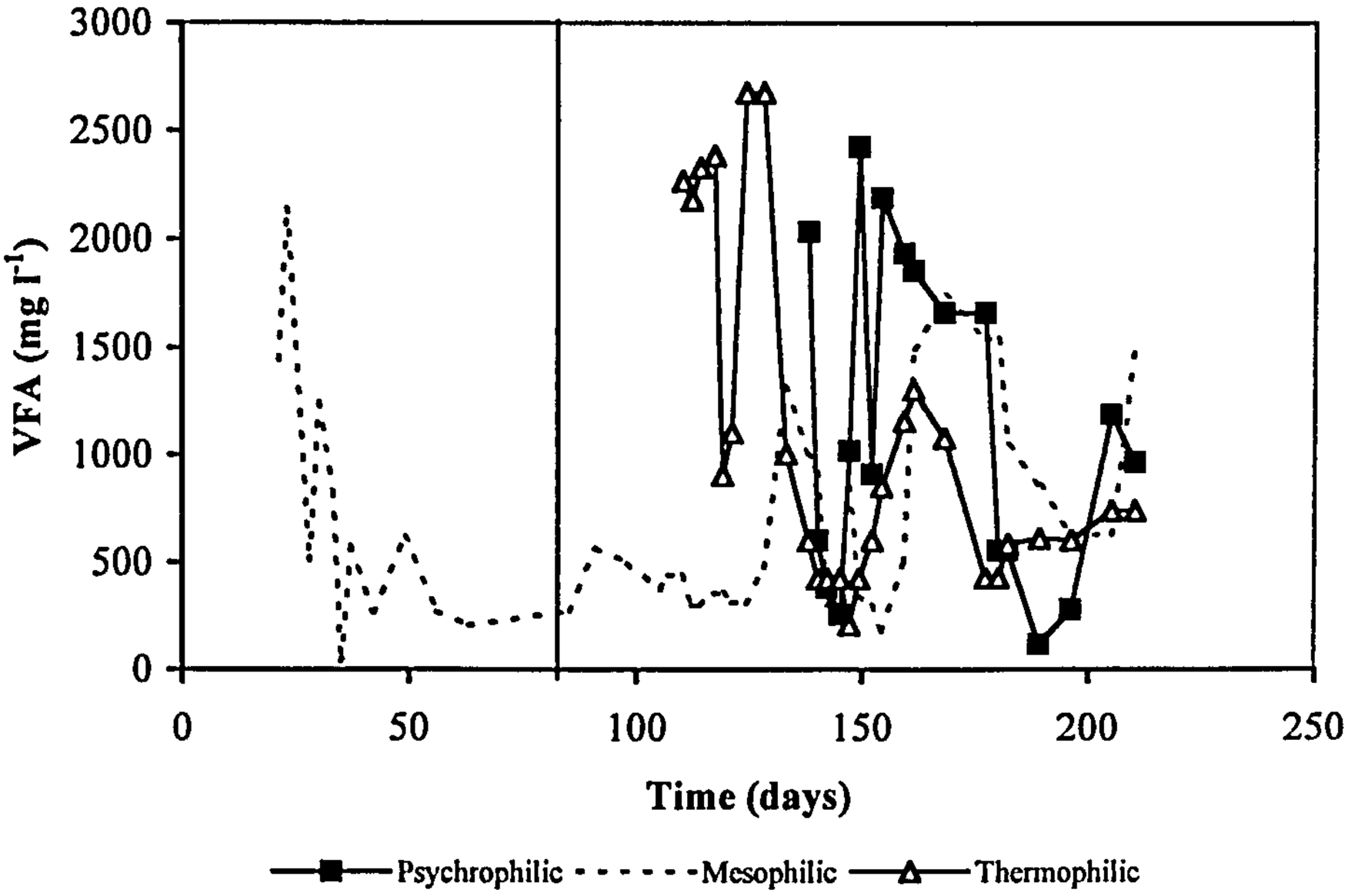


Figure 5.7. Effluent VFA concentrations for all three reactors.

Despite the considerable variation in feed composition, a one way ANOVA revealed that effluent reactive P concentrations were in statistically significantly lower than those of the feed (where $n = 69$, $F_{3, 88} = 19.02$, $P < 0.05$), with average removals at HRT 23.33 days of 76% for the psychrophilic digester, 83% for the mesophilic digester and 55% for the thermophilic system (Figure 5.8). The fate of P entering the digesters was not determined, but it is likely that it was either incorporated into biomass, adsorbed onto suspended solids or formed insoluble precipitates with metallic ions (Carliell and Wheatley, 1997). Effluent TAN concentrations (Figure 5.9) were consistently higher than feed concentrations for all three digesters, average values rising from *c.* 50 ± 25 mg l^{-1} for the feed to *c.* 500 ± 170 mg l^{-1} for the effluents; these increases were probably due to ammonia formation by deamination of proteins present in the waste.

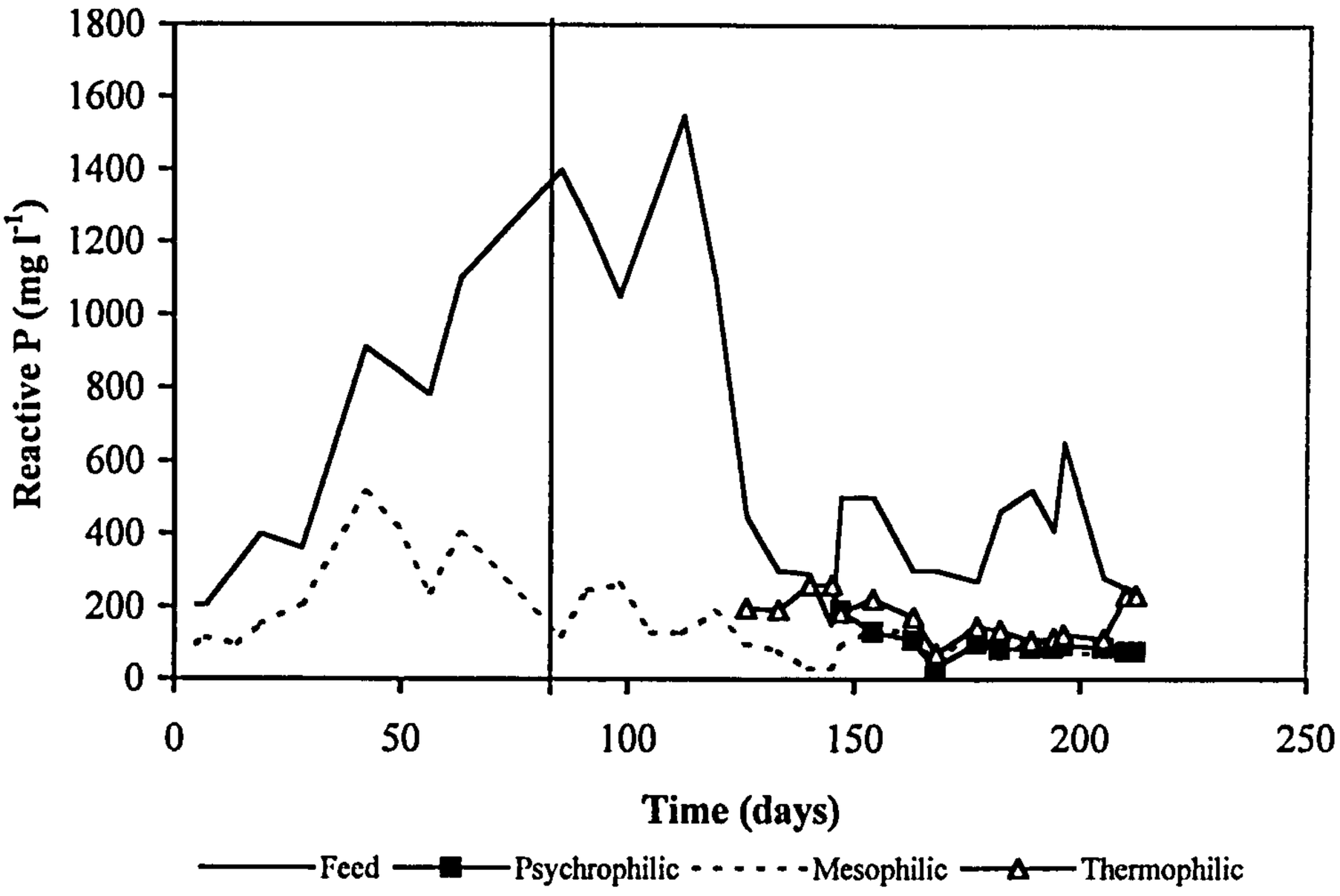


Figure 5.8. Feed and effluent reactive P concentrations.

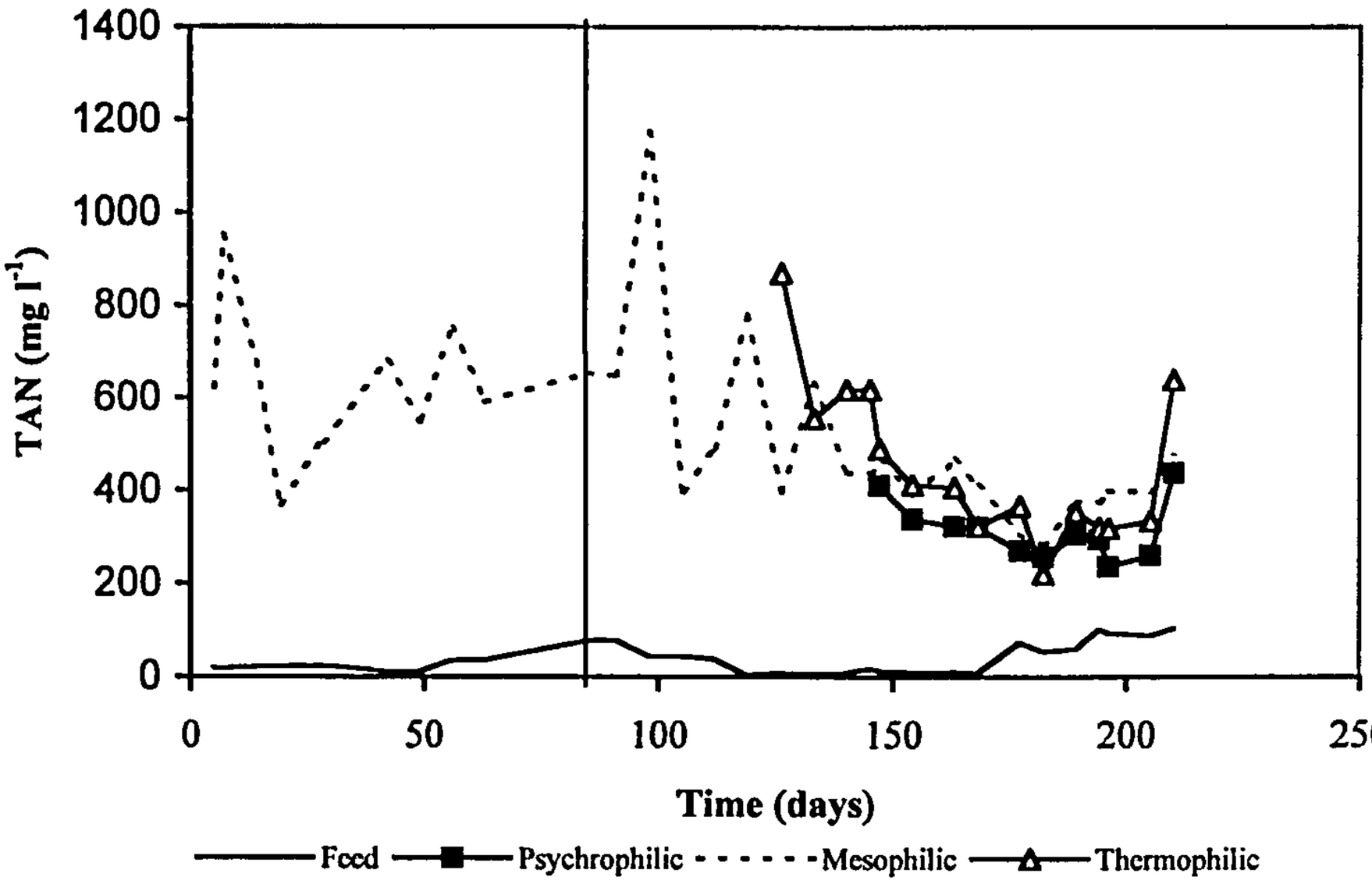


Figure 5.9. Feed and effluent TAN concentrations for all three reactors.

5.1.3. Discussion

Although all digester performances at 23.33 days HRT were reasonably stable, there were still considerable variations in parameters such as feed strength, effluent COD and biogas production, making calculation of quantitative performance indicators such as percentage COD removal and biogas yield difficult. Nevertheless, an approximation can be obtained by comparing average values for TS, SS, COD and biogas production over the period concerned. It is assumed that any observed removal of solids or COD was due to biodegradation rather than sedimentation and accumulation within the digesters, which is a reasonable assumption given the relatively constant levels of TS and SS in each digester in the latter stages of the study (Figure 5.1).

It is evident that the digesters removed significant proportions of the TS, SS, COD and BOD fed (Table 5.1). The values for mesophilic solids removal are possibly low as the peak values measured on days 42, 98 and 117 were included in the calculation. These values may be unrepresentative as discussed earlier, and if they are discarded from the data set, TS and SS removal for the mesophilic digester at HRT 23.33 days rise to 69% and 80% respectively.

Surprisingly, TS, SS and COD removals for the psychrophilic digester appear as good as, if not better, than those for the other two digesters, although BOD removal was poorer with higher residual BOD concentrations (Table 5.1). This result is consistent with the higher effluent BOD/COD ratios observed in the psychrophilic system. Compared to the theoretical maximum (0.35 m^3 CH_4 (at 0°C and 1 atm) kg^{-1} COD removed (COD_{rem}), both the mesophilic (0.21 m^3 CH_4 kg^{-1} COD_{rem}) and thermophilic (0.20 m^3 CH_4 kg^{-1} COD_{rem}) digesters performed well (excluding period of initial reactor start up, days 85 - 160). The

CH_4 yield for the psychrophilic system was very low (0.07 m^3 CH_4 kg^{-1} COD_{rem}). This result cannot be explained in terms of temperature or pH dependent gas solubility effects. It may be that in the psychrophilic digester, a significant proportion of the C removed left the system in the form of a gas which was not detected in the biogas analysis, although this seems unlikely.

The comparable removal rates of solids and oxygen demand (COD and BOD) for the psychrophilic reactor (which had no heating coil) may be explained by the method employed to remove effluent from the mesophilic and thermophilic digesters. Prior to extraction of effluent, the mixing stirrer within the digester was switched off to allow large SS to settle allowing the supernatant only to be removed. During this period of sedimentation, however, heat was still applied to the periphery of the reactor resulting in a temperature gradient. It may be reasoned that as the core of the reactor loses heat, probably due to natural convection through the floor or roof of the reactor vessel, heat transfer from the periphery of the vessel could have resulted in thermal circulation within the digester. This would cause smaller particles to rise and circulate around the reactor and thus readily be removed in the effluent. The observed poor solids removal rate of the thermophilic reactor concurs with this hypothesis.

Comparison of the TS and SS concentrations for individual samples (Mixed liquor, feed and effluent data) shows that there was a strong correlation between these parameters. Figure 5.10 illustrates the relationship between TS and SS for all cases in which both were measured, the data set including points from analyses of feeds, digester effluents and digester mixed liquors. Curve fitting gives a linear correlation with an R^2 value over 0.99. The fact that the slope is also close to unity (0.93) suggests that the samples had a fairly constant level of soluble solids (*c.*1,400 mg l^{-1}) with the remainder of the TS made up of SS, so that the TS concentration of any given sample was dependent primarily on

the SS level. As feed TS was generally much higher than the soluble solids concentration (i.e. TS minus SS), it is clear that most of the organic pollutant load present in the feed was associated with the solid phase.

If it is postulated that anaerobic digestion proceeded by solubilisation of SS followed by further metabolism of the soluble organic products formed, the results of the TS and SS correlation would be consistent with a relatively slow first step producing soluble organics which were then rapidly degraded, giving a reasonably constant low level of soluble solids. This mechanism would be expected since hydrolysis and hence solubilisation of the solid phase is generally held to be the rate-limiting step in anaerobic solids digestion (Pfeffer, 1979; Wang et al., 1997; Quarmby et al., 1999). Further evidence for this hypothesis comes from the fact that BOD and soluble COD concentrations were generally much lower than total COD values.

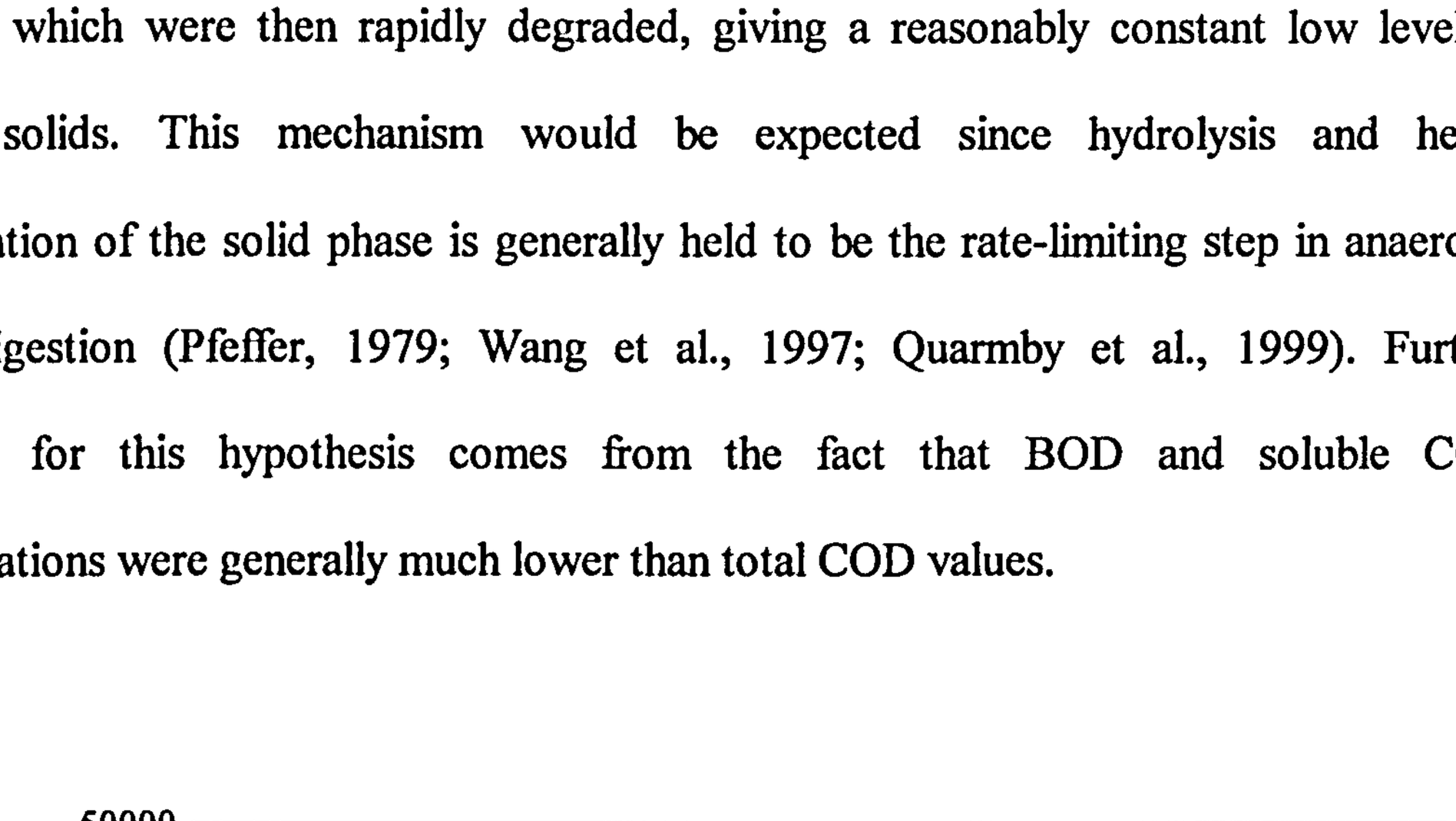


Figure 5.10. TS and SS correlation.

5.2. PATHOGEN INACTIVATION

5.2.1. Experimental Procedure

5.2.1.1. Thermophilic anaerobic reactor.

Two thermophilic anaerobic reactors as described in *Section 3.2.1* operating at a HRT of 23.33 days and an organic loading rate of *c.* 0.34 kg COD m^{-3} d^{-1} were used to examine the potential for destruction of *Y. ruckeri*. Aquaculture waste effluent feed for each digester was inoculated with *Y. ruckeri* to yield a concentration of 1.25×10^6 cells ml^{-1}. Inoculated feed was fed to the digesters and subsequent supernatant effluent removed from the digester, as described in *Section 3.3.2*, was examined for the survival of *Y. ruckeri* (*Section 3.5.1*). Aquaculture waste digester liquor and feed samples were also examined for the presence of the pathogen.

5.2.1.2. Thermophilic bioassays

To examine the effect of temperature on *Y. ruckeri*, duplicate nutrient broth solutions (50 ml each) were placed in a shaking water bath at 55°C and inoculated with *Y. ruckeri* to yield a solution concentration of 1.4×10^8 cells ml^{-1}. Duplicate samples for the determination of pathogen survival were taken at a variety of time intervals after inoculation namely, 0, 10, 20, and 30 minutes, and 24 h. A control sample of nutrient broth at room temperature was also analysed. All samples were analysed for the presence of *Y. ruckeri*, as described in *Section 3.5.3*.

5.2.1.3. Psychrophilic bioassays

The effect of psychrophilic digester sludge on the destruction of *Y. ruckeri* was examined using 50 ml of psychrophilic digester sludge. The digester sludge was inoculated with *Y. ruckeri* to yield a concentration of 1.7×10^6 cells ml^{-1}. Duplicate psychrophilic sludge samples were inoculated with the pathogen. Control samples were also monitored with un-inoculated psychrophilic sludge and a nutrient broth solution inoculated with *Y. ruckeri*. Duplicate samples of each bioassay were analysed at time intervals of 0, 10, 20, and 30 minutes and all samples were examined for the presence of the pathogen, as described in *Section 3.5.3*.

5.2.2. Effect of Temperature and Anaerobic Sludge on Fish Pathogen

The thermophilic anaerobic digestion of aquaculture waste inoculated with *Y. ruckeri* appeared to inactivate the pathogen completely within 2 days (Time period between each feeding/sludge removal of batch digester, *Section 3.3.2*). The presence of the pathogen on the selective media (*Section 3.5.3*) from digester liquor and effluent samples was not observed (too few to count (TFTC)). Positive controls (at room temperature, *c.* 22°C) of pathogen in nutrient broth showed the exponential growth of the pathogenic bacteria (Table 5.2). Background controls of digester liquor and aquaculture waste before inoculation did however reveal the presence of microorganisms characteristic of the yellow fluorescent growth indicator expected from *Y. ruckeri* on ribose ornithine deoxycholate medium. Throughout the experiment using the thermophilic reactors, these microorganisms were observed during analysis of digester liquor, effluent and control feed samples. Type 1 *Y. ruckeri* antiserum revealed that these were not *Y. ruckeri.*

Bioassay experiments also showed the complete inactivation of the pathogen (Table 5.2). Nutrient broth solution at 55°C inoculated with the pathogen demonstrated that high temperature resulted in the total decay of *Y. ruckeri*. The growth of any microorganisms on the media was not observed (TFTC). Similarly, assays containing psychrophilic digester sludge resulted in the complete destruction of *Y. ruckeri*. Positive controls for both assays were successful resulting in the growth *Y. ruckeri* (too numerous to count (TNTC)). This was confirmed by Type 1 *Y. ruckeri* antiserum.

Table 5.2. Summary of results for the survival/ destruction of *Y. ruckeri* at high and low temperature digestion.

		Presence of *Y. ruckeri*	**Concentration of *Y. ruckeri***
Thermophilic digester	Background	No	N A
	Control	Yes	TNTC
	Sample	No	Total death occurred within 2 days (TFTC)
Thermophilic assay	Background	N A	N A
	Control	Yes	TNTC
	Sample	No	Total death occurred < 1 min
Psychrophilic assay	Background	No	N A
	Control	Yes	TNTC
	Sample	No	Total death occurred < 1 min

5.2.3. Discussion

Y. ruckeri has an optimum growth temperature of 24°C (Austin and Austin 1999). Similarly, the optimum growth temperature of many pathogenic organisms (*Enterobacteriaceae*) ranges from 35 - 42°C. Drnevich and Smith (1975) showed that long exposure to temperatures above 45°C is lethal for these microbes. They also demonstrated that at 60°C, destruction of the pathogens was extremely rapid, with *Salmonella* spp. falling in number to below detectable limits within a few hours. Although both the thermophilic and psychrophilic digester sludge appeared to inactivate the pathogen, the rate of total kill seems unlikely. Samples were monitored within 1 min in order to evaluate time 0 (Time 0 = initial concentration *Y. ruckeri* in assay immediately after spiking), which revealed the presence of no pathogen for both the thermophilic and psychrophilic sludge. It would not be expected to achieve total inactivation of the pathogenic organism within such a short period of time.

Farrah and Bitton (1983) reported a decay rate of 0.6 – 0.9 $\log_{10}$ ml^{-1} day^{-1} for *Salmonella* and *Escherichia coli* spp. within a mesophilic anaerobic digester operating at a HRT of 15 days. Similarly, Kearney et al. (1993) showed the destruction of pathogenic bacteria such as *Salmonella* in cattle slurry fed to a mesophilic reactor (28°C) to reduce 90% of pathogens in 34.5 days. *Salmonella* spp. (concentration of 1.57×10^{10}) applied to biosolids, and stored under anaerobic conditions, had a decay rate of 1.13 $\log_{10}$ day^{-1} at 49°C with maximum time for destruction to below detectable limits of 7 days. When the temperature was decreased to 38°C, destruction to below detectable limit was at least 20 days and at 22°C greater than 62 days (Ahmed and Sorensen, 1995). Although stored biosolids cannot be strictly compared to the optimised conditions within an anaerobic reactor, it demonstrates the significance of increased temperature under anaerobic

conditions in relation to the inactivation of pathogenic bacteria. Liltved et al. (1995) demonstrated the use of ozonation for the destruction of common fish bacterial diseases. It was shown that a 4 $\log_{10}$ reduction of *Y. ruckeri* could be achieved within 180 s at a residual ozone concentration of 0.15 – 0.2 mg l^{-1}. However, the almost instantaneous destruction of pathogenic bacteria to below detectable limits under anaerobic conditions has not previously been reported in literature.

The almost instantaneous destruction (8 $\log_{10}$ within 120 s) of *Y. ruckeri* when inoculated in a nutrient broth solution at 55°C during this study appears to indicate the positive influence of temperature in terms of the inactivation of *Y. ruckeri* when compared to the control sample at room temperature. Similarly, the destruction of *Y. ruckeri* when inoculated into psychrophilic sludge was relatively instantaneous. This may have been due to the initial stress caused by the anaerobic sludge and thus failure to grow on the selective medium. Although the medium was theoretically selective to the growth of *Y. ruckeri*, unknown cultures of bacteria were observed. Type 1 *Y. ruckeri* antiserum revealed that these cultures were not *Y. ruckeri* organism. This may also have resulted in the out competing of already stressed *Y. ruckeri* organisms from culturing on the selective medium.

Alternatively, the presence of *Y. ruckeri* may not have been detected due to adsorption of the pathogen to particulate material in the digester sludge. Kearney et al. (1993) reported the viable numbers of *Salmonella* spp. to be greater in the solid fraction than in the liquid fraction of anaerobic digester sludge. In contrast, the same study reported the viable numbers of *Campylobacter jejuni* were greater in the liquid fraction than in the solid fractions demonstrating that adsorption to solids was species dependent. *Y. ruckeri* is strongly hydrophobic (Austin and Austin, 1999) and has been shown to have a high ability to adhere to solid surfaces (Coquent et al. 2001). In this study, samples for growth

on selective media were taken from the liquid fraction only. It is possible that the organism was bound to particulate material after inoculation and therefore growth on the selective media would not be expected. Although bound to the solid fraction, inactivation of the organism would still be expected to occur but evaluation of this would require sampling of digester solid material.

It was not possible to further evaluate the cause of pathogen inactivation within such a short time period. Further research is therefore needed to confirm and evaluate the results. It is however evident from this study and previous studies reported in literature that the use of anaerobic digestion, particularly under thermophilic conditions, is a competent means for pathogen destruction.

Chapter 6

Enhancement of the Anaerobic Digestion of Aquaculture Effluents

6.1. NUTRIENT SUPPLEMENTATION

6.1.1. Experimental Procedure

Nutrient enhancement (*Section 2.10.1*) of the anaerobic digestion of aquaculture effluents at psychrophilic, mesophilic and thermophilic temperatures at a HRT 23.33 days was examined using reactors as detailed in *Section 3.3.1*. Ca, Fe and Mg at a concentration of 10 mg l^{-1} were added to the aquaculture waste on every feeding (3 times per week). A trace element solution at a concentration of 1 ml l^{-1} was also added (Table 6.1). The anaerobic digesters were operating under stable conditions as a result of the previous experimental work described in *Section 5.1*. In order to examine the influence of nutrient supplementation on the digestion process, days 166 - 212 from the previous experiment were used as a control (no nutrient addition) and became known as days 0 - 46 in this experiment. Duration of the experiment, including data from previous experiment, was 115 days with the addition of nutrients commencing on day 47.

Table 6.1. Composition and concentration of nutrient supplemented to the digester feed (Angelidaki et al. 1990).

Component	Concentration (mg l^{-1})
$MgS0_4.7H_2O$	102.50
$CaCl_2.2H_2O$	36.75
$FeSO_4.7H_2O$	18.29
Trace elements:	
$NiSO_4.6H_2O$	106.00
$MnCl_2.4H_2O$	110.00
$ZnSO_4.7H_2O$	100.00
H_3BO_3	104.00
$CoCl_2.6H_2O$	48.00
$H_3PO_4.12MoO_3.24H_2O$	48.00
$CuSO_4.5H_2O$	20.00
$AlCl_3.6H_2O$	22.00
EDTA	1000.00
$Na_2ScO_2.5H_2O$	100.00

6.1.2. Effect of Nutrient Addition to the Digestion Process

Digester solids levels remained relatively constant for both mesophilic and thermophilic temperatures (Figure 6.1). The relative stability of the mixed liquor digester solids confirms digestion of organic material as opposed to sedimentation of the solids within the reactor. However, there was a continuous increase in psychrophilic digester solids between days 35 and 115 from 13,000 mg l^{-1} to 27,000 mg l^{-1} respectively. In contrast to the high temperature digesters, this is an indication of sedimentation within the reactor, rather than digestion of organic material.

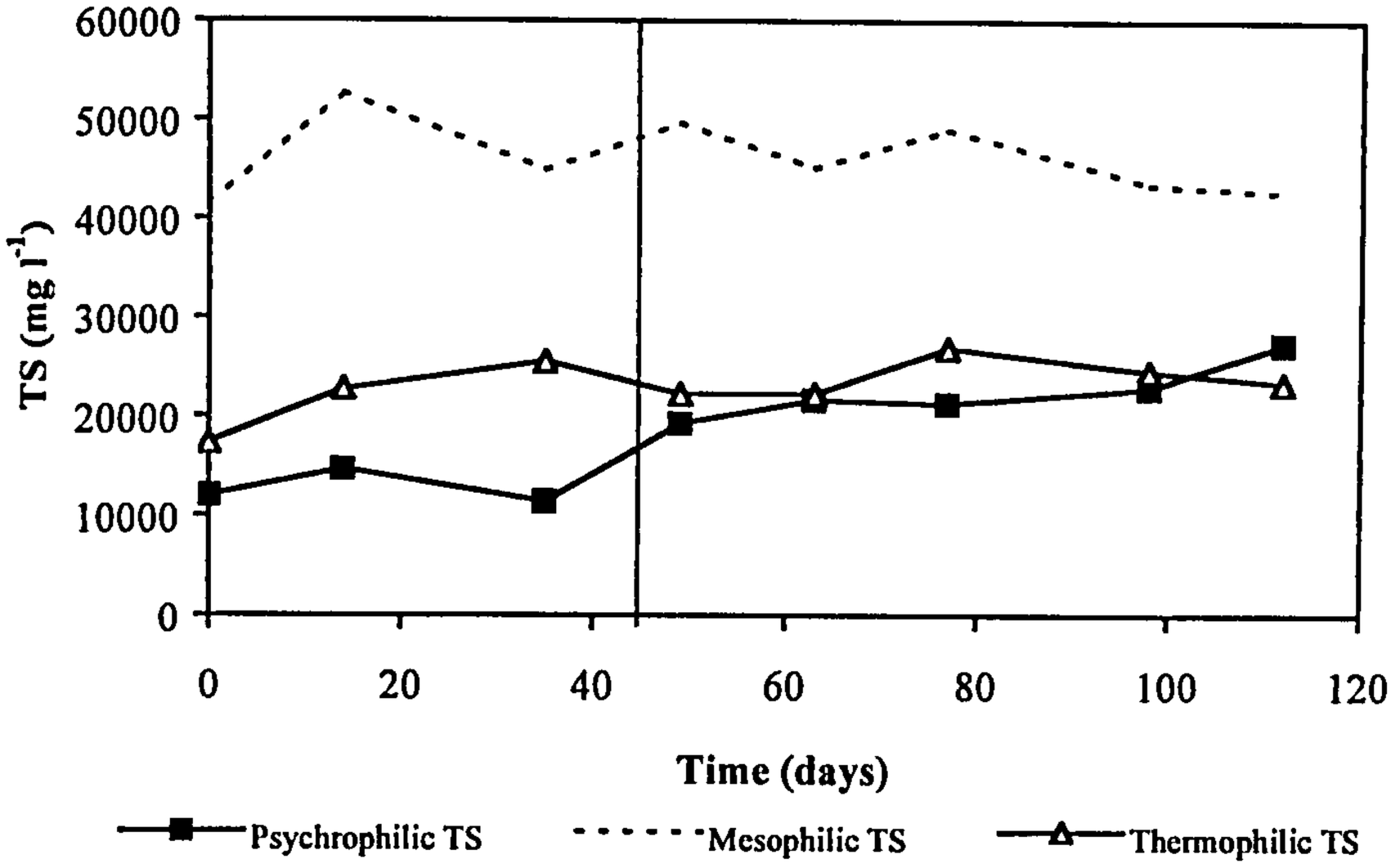


Figure 6.1. Digester mixed liquor TS for all three operating temperatures.

Effluent TS and SS values during the period of nutrient supplementation (days 47 - 115) show no improvement from those during the previous non-supplemented period (Figures 6.2 and 6.3). Statistical analysis (one-way ANOVA) revealed that there was no significant difference between removal efficiencies of TS at psychrophilic (where $n = 15$, $F_{1, 13} = 0.164$, $P < 0.05$), mesophilic (where $n = 15$, $F_{1, 13} = 0.792$, $P < 0.05$), and thermophilic (where $n = 15$, $F_{1, 13} = 0.294$, $P < 0.05$) operating temperatures as a result of nutrient supplementation. Similarly, the difference in SS mean removal efficiencies with as a result of nutrient addition was not statistically significant psychrophilic (where $n = 14$, $F_{1, 12} = 0.338$, $P < 0.05$), mesophilic (where $n = 14$, $F_{1, 12} = 1.58$, $P < 0.05$), and thermophilic (where $n = 14$, $F_{1, 12} = 1.54$, $P < 0.05$) operating temperatures. TS were on average *c.* 3,000 ± 440 mg l^{-1}, 2,600 ± 386 mg l^{-1} and 3,000 ± 432 mg l^{-1} for the psychrophilic, mesophilic and thermophilic digester effluents respectively for both periods of digestion.

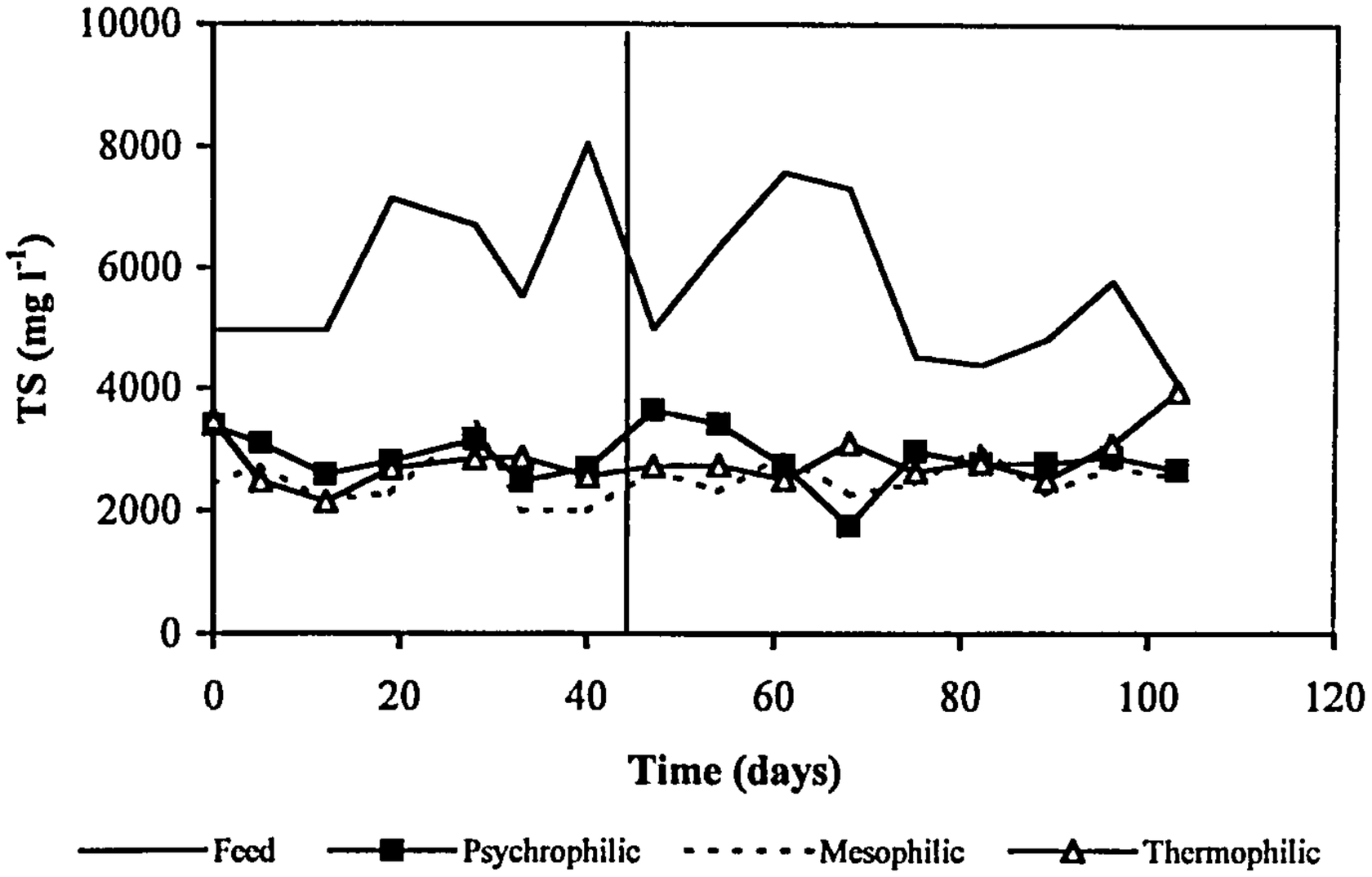


Figure 6.2. Digester Feed and effluent TS concentrations.

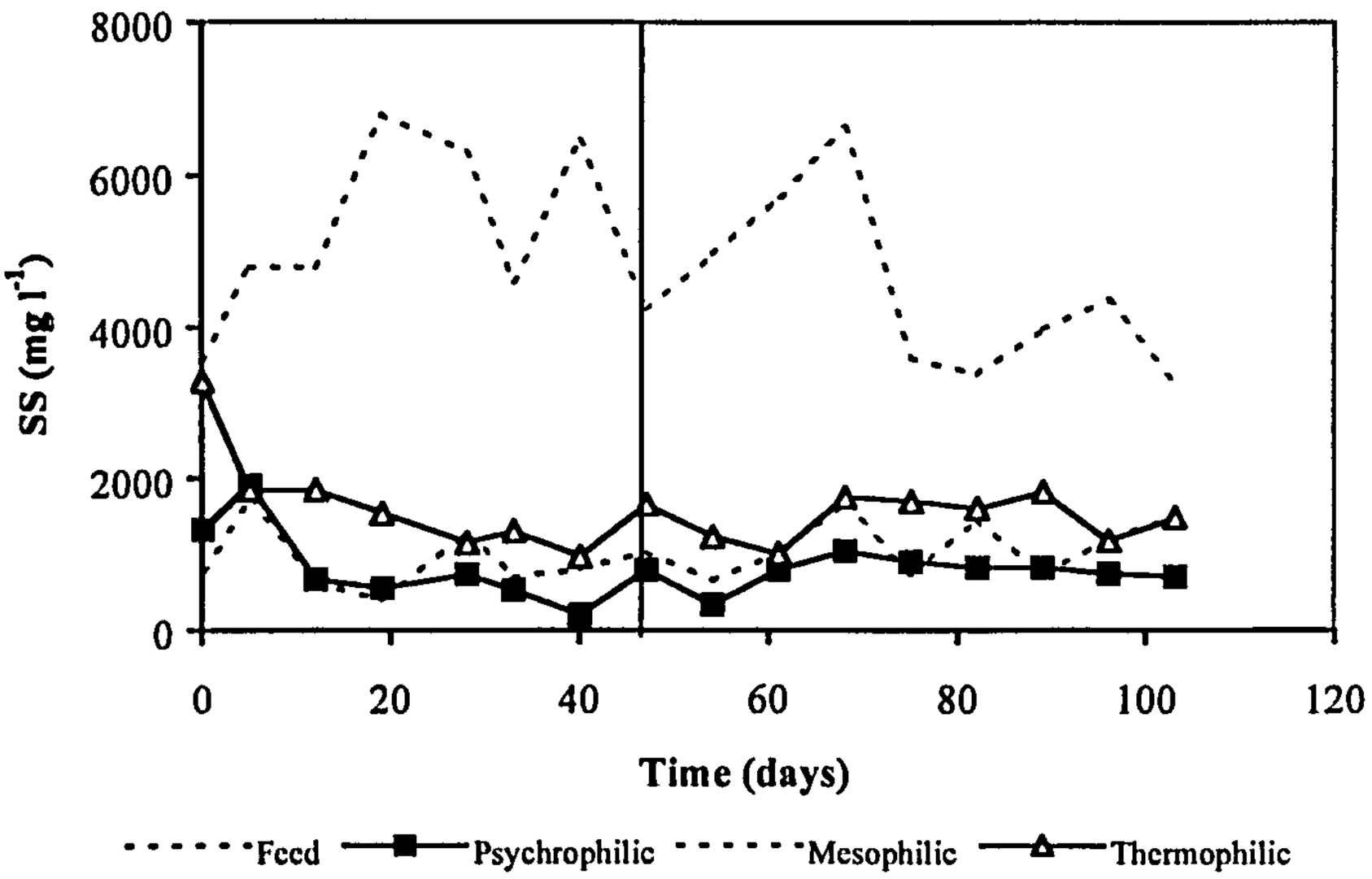


Figure 6.3. Digester feed and effluent SS concentrations.

Feed COD data varied slightly throughout the experiment (Figure 6.4). This was due primarily to the nature of the waste and the degree of difficulty involved in analysing a representative sample during the COD assay test. Effluent COD samples being more liquid and less agglomerated in nature were easier to analyse in terms of representative samples. The effluent COD values were consistently lower than those of the feed, the

only exception being the value on day 89 for the psychrophilic effluent. This may be attributed to sampling error through poor settling of sludge in the psychrophilic reactor and therefore large solid particles being drawn out with the effluent sample. The addition of nutrients in the second period did however result in a greater COD removal. For mesophilic and thermophilic digestion, there was a 5 - 10% increase in the removal of COD. The psychrophilic reactor would have had a similar COD removal rate if the experimental error on day 89 was excluded from the calculations.

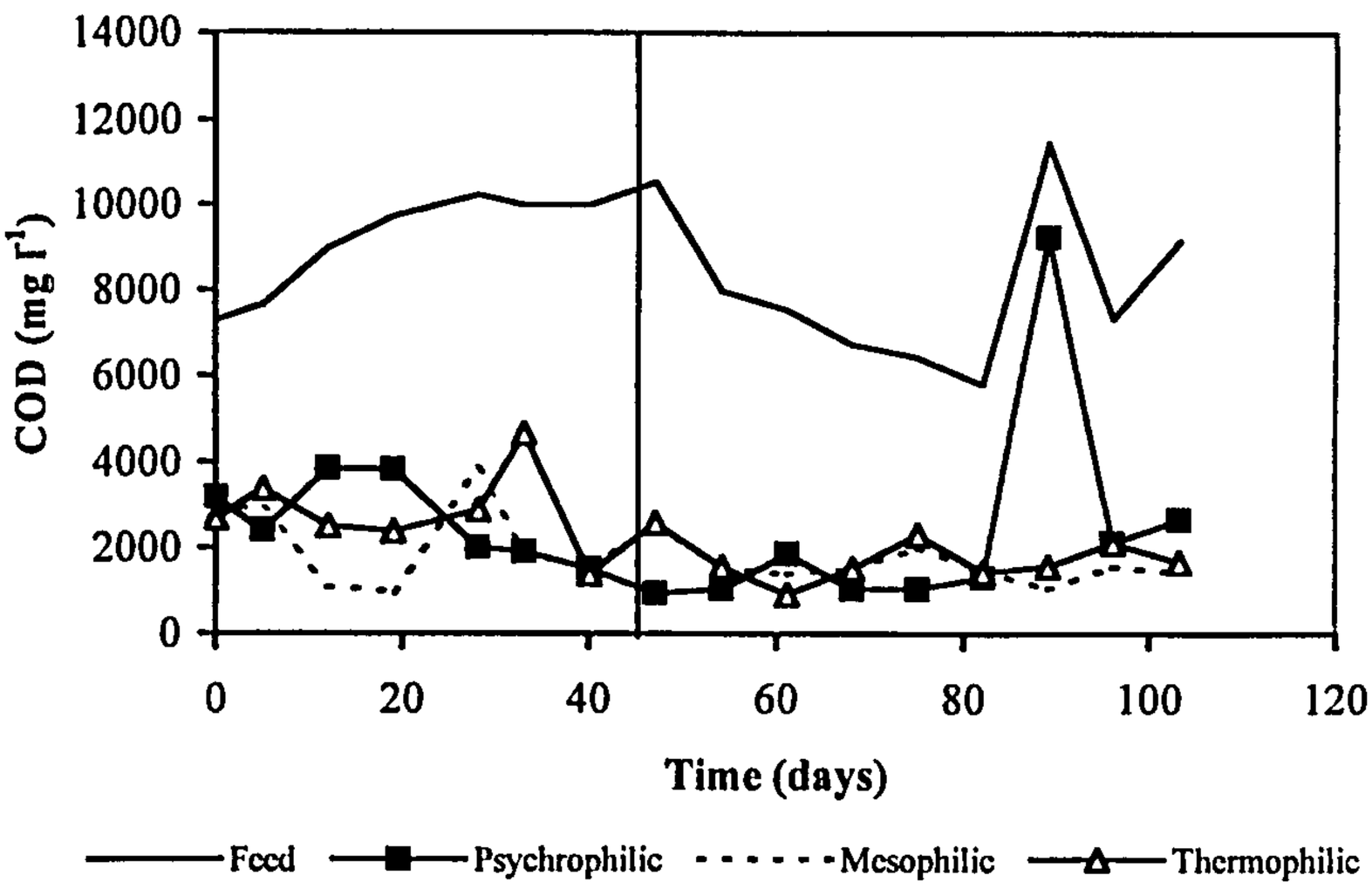


Figure 6.4. Digester feed and effluent COD concentrations.

The trace element period saw no significant increase in the reduction of BOD. Statistical analysis (one-way ANOVA) revealed that removal efficiencies for the psychrophilic (where $n = 14$, $F_{1, 12} = 1.75$, $P < 0.05$), mesophilic (where $n = 14$, $F_{1, 12} = 1.54$, $P < 0.05$) and thermophilic (where $n = 14$, $F_{1, 12} = 2.52$, $P < 0.05$) reactors in each case were not significantly different with as a result of nutrient addition, being 83, 95 and 90% respectively.

Biogas production from days 0 - 46 and 47 - 115 was on average 0.15 ± 0.08, 0.45 ± 0.15, and 0.42 ± 0.12 l day^{-1} and 0.14 ± 0.05, 0.41 ± 0.13, and 0.38 ± 0.14 l day^{-1} for the psychrophilic, mesophilic and thermophilic reactors respectively. The difference in mean biogas production yield between the two feeding periods, where $n = 116$ in each case, was not statistically significantly (one-way ANOVA) for the psychrophilic ($F_{1, 114} = 1.09$, $P < 0.05$), mesophilic ($F_{1, 114} = 1.15$, $P < 0.05$) and thermophilic ($F_{1, 114} = 1.54$, $P < 0.05$) reactors. On analysis, biogas composition for both periods was similar with an average CH_4 content of 60%. However, on calculation of the average yield of CH_4 per kilogram of COD removed, there was slightly less efficiency in conversion for both mesophilic and thermophilic reactors with 0.19 and 0.17 m^3 CH_4 kg COD_{rem}^{-1} respectively (Table 6.2). There was no change in the psychrophilic CH_4 conversion yield.

Table 6.2. Comparison of digester performance with and without nutrient supplementation. Results of nutrient addition in brackets.

	Psychrophilic	**Mesophilic**	**Thermophilic**
% TS reduction	52 (51)	58 (56)	54 (51)
% SS reduction	84 (84)	83 (78)	68 (69)
% COD reduction	72 (72)	77 (80)	70 (80)
% BOD reduction	83 (83)	95 (95)	90 (90)
% Reactive P reduction	76 (85)	76 (84)	67 (76)
m^3 CH_4 $COD_{rem}^{-1}day^{-1}$	0.07 (0.07)	0.21 (0.19)	0.20 (0.17)

VFA concentrations were analysed in each digester effluent stream. Thermophilic and mesophilic VFA concentrations were generally below 1,000 mg l^{-1}. Psychrophilic VFA concentrations were slightly higher than those of the other reactors averaging *c.* 1,100 mg l^{-1}. VFA was predominately in the form of acetic and propionic acid.

Reactive P was analysed in both the feed and effluent streams. Typically, there was a reduction of 76% for both the psychrophilic and mesophilic reactors and 67% for the thermophilic reactor. As discussed in *Section 5.1.2* it was expected that the fate of reactive P was adsorption onto SS or incorporation into digester biomass. TP (insoluble and soluble) was also examined periodically in the digester feed, liquor and effluent streams (Table 6.3). It was evident that a large proportion of the TP entering the system was not incorporated into the digester biomass and particulate material, resulting in the washout of TP from the digester in the effluent stream. Therefore, only a small quantity of TP was assimilated into the system, this presumably being the soluble fraction.

Table 6.3. Digester Feed, Liquor and Effluent TP concentrations.

Day	Feed TP ($mg\ P\ g^{-1}$)	Digester	Digester Liquor TP ($mg\ P\ g^{-1}$)	Digester Effluent TP ($mg\ P\ g^{-1}$)
82	26.83	Psychrophilic	29.04	27.32
		Mesophilic	21.94	25.40
		Thermophilic	20.11	24.54
103	18.42	Psychrophilic	24.89	19.56
		Mesophilic	19.74	18.65
		Thermophilic	18.66	12.18

Throughout the study, pH and Alk of the anaerobic digesters at all three operating temperatures were stable at a range of 7.0 - 7.2 and 2,500 - 3,500 mg $CaCO_3$ l^{-1} respectively. Total ammonia nitrogen (TAN) was also monitored and did not exceed 400 mg l^{-1}.

6.1.3. Discussion

The performance of the anaerobic reactors at all three operating temperatures was successful in the digestion of aquaculture waste effluents. An examination of the mesophilic and thermophilic digester mixed liquor solids contents confirms that removal of solids and hence COD and BOD was due to digestion of the feed material and not simply sedimentation within the reactors (Figure 6.1). Conversely, the digester mixed liquor solids content of the psychrophilic reactor shows evidence of solids accumulation rather than digestion. Nutrient supplementation did not appear to make any statistically significant improvement to the digestion process. There was a negligible increase in COD removal of 1 - 3% for both the psychrophilic and mesophilic digesters. There was however a slightly greater increase in the removal of COD by the thermophilic digester of *c.* 10%. This cannot be explained in terms of greater TS removal or greater utilisation by microorganisms, as there was no related biogas increase. This is confirmed by calculation of the CH_4 yield, which shows no improvement in process efficiency.

The removal efficiency (Table 6.2) of SS, and oxygen demand (both BOD and COD) in the mesophilic and particularly the thermophilic temperature range appear to be relatively poor in comparison with the psychrophilic reactor. If is it hypothesised that the greater the conversion of organic material to CH_4 then the greater the efficiency of the process, it may therefore be considered that the high temperature reactors were more efficient. The cumulative biogas production rate (Figure 6.5) shows that the rate of biogas production by both the mesophilic and thermophilic reactors was far greater than that of the low temperature digestion process. Calculation of the conversion yields of COD to CH_4 further confirms the greater efficacy of the high temperature processes (Table 6.2).

Mixed liquor TS results also indicate sedimentation within the psychrophilic reactor, rather than digestion of the aquaculture waste. The comparable removal rates of solids and oxygen demand for all three reactors may also be explained by the method employed to remove effluent from the reactors as described in *Section 3.3.2*, the effect of the heating system employed on the sedimentation of particulate material prior to effluent removal from the reactor.

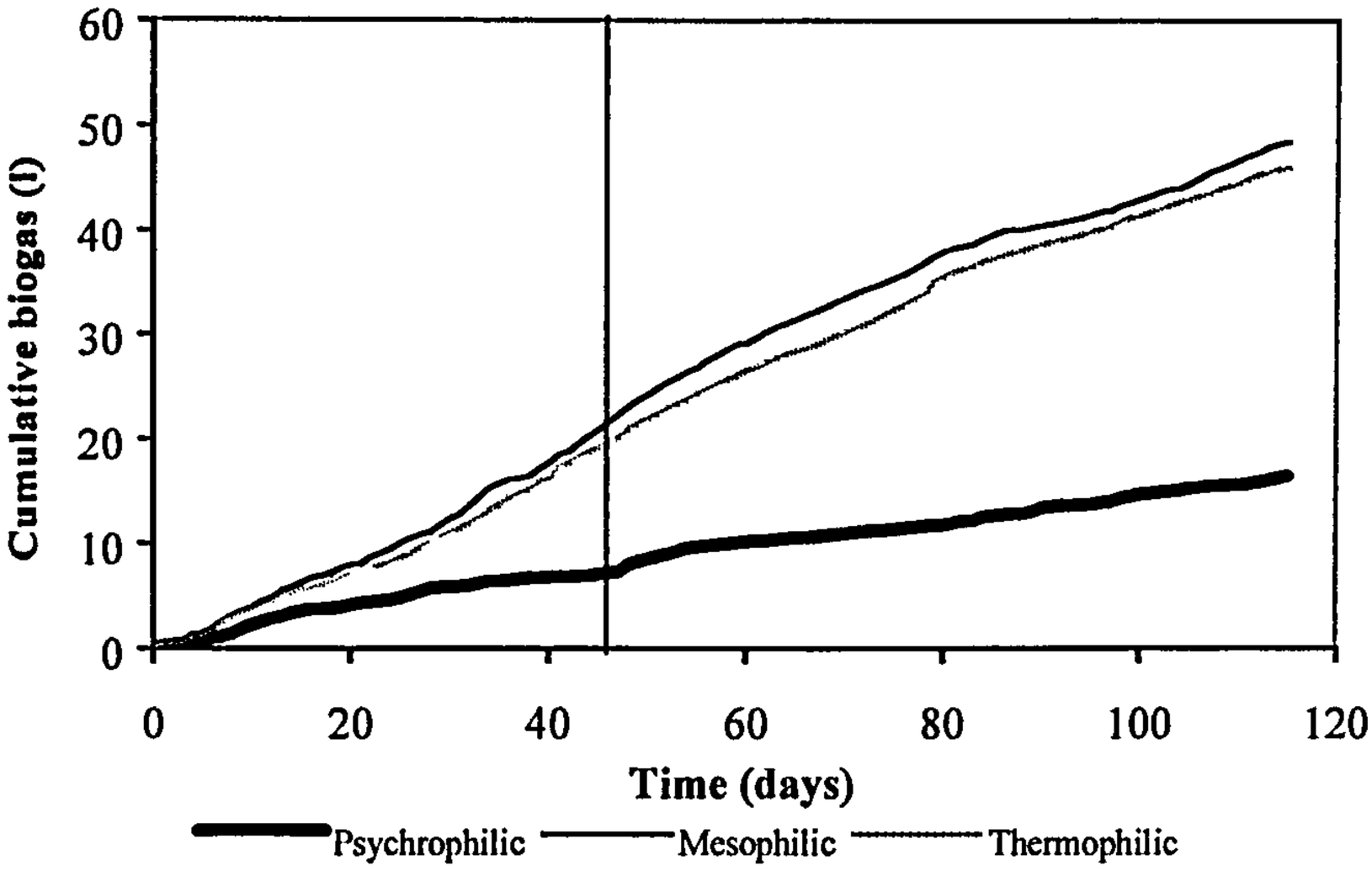


Figure 6.5. Cumulative biogas production for all three reactors.

The removal of reactive P from the feed was high, ranging from 67 - 76% for all three reactors. The majority of feed TP (organic and reactive P) leaves in the effluent stream (Table 6.3). As with the previous experiment (*Section 5.1.2* and Table 6.2), thermophilic reactive P removal was lower than both mesophilic and psychrophilic digesters, which had comparable percentages for P removal. This is a similar trend to that of SS and COD removal for all three reactors. Reactive P occurs in both dissolved and suspended form (APHA, 1995). If the hypothesis that poor settling occurs within the thermophilic reactor (as discussed in *Section 5.1.3*) is correct, it would explain the lower levels of P removal from the thermophilic digester.

In *Section 2.10.2*, it was generally regarded that the rate-limiting step during the anaerobic treatment process is the initial phase of bacterial breakdown of solid matter to soluble material for metabolism. An examination of the solids data from this experiment also reveals a strong correlation between TS and SS (Figure 6.6). Comparing TS and SS from feed, mixed liquor and effluent data, there is a linear correlation between the two parameters, and the gradient of line was approaching unity, at a value of 0.96. As with data from the previous experiment, this suggests that only a small concentration of TS was soluble in nature and SS was the dominant fraction. The results of the TS and SS correlation are therefore consistent with the recognition of hydrolysis being the rate-limiting step. The relatively low values for soluble COD and BOD in comparison to total COD also confirm this. Pre-treatment of the aquaculture effluents may therefore be required in order to improve the initial stage of hydrolysis and hence the anaerobic digestion process. This may be achieved by employing a means of reducing the particle size of the SS (*e.g.* thermal or chemical pre-treatment, ultrasonication) making it easier for bacteria to break down.

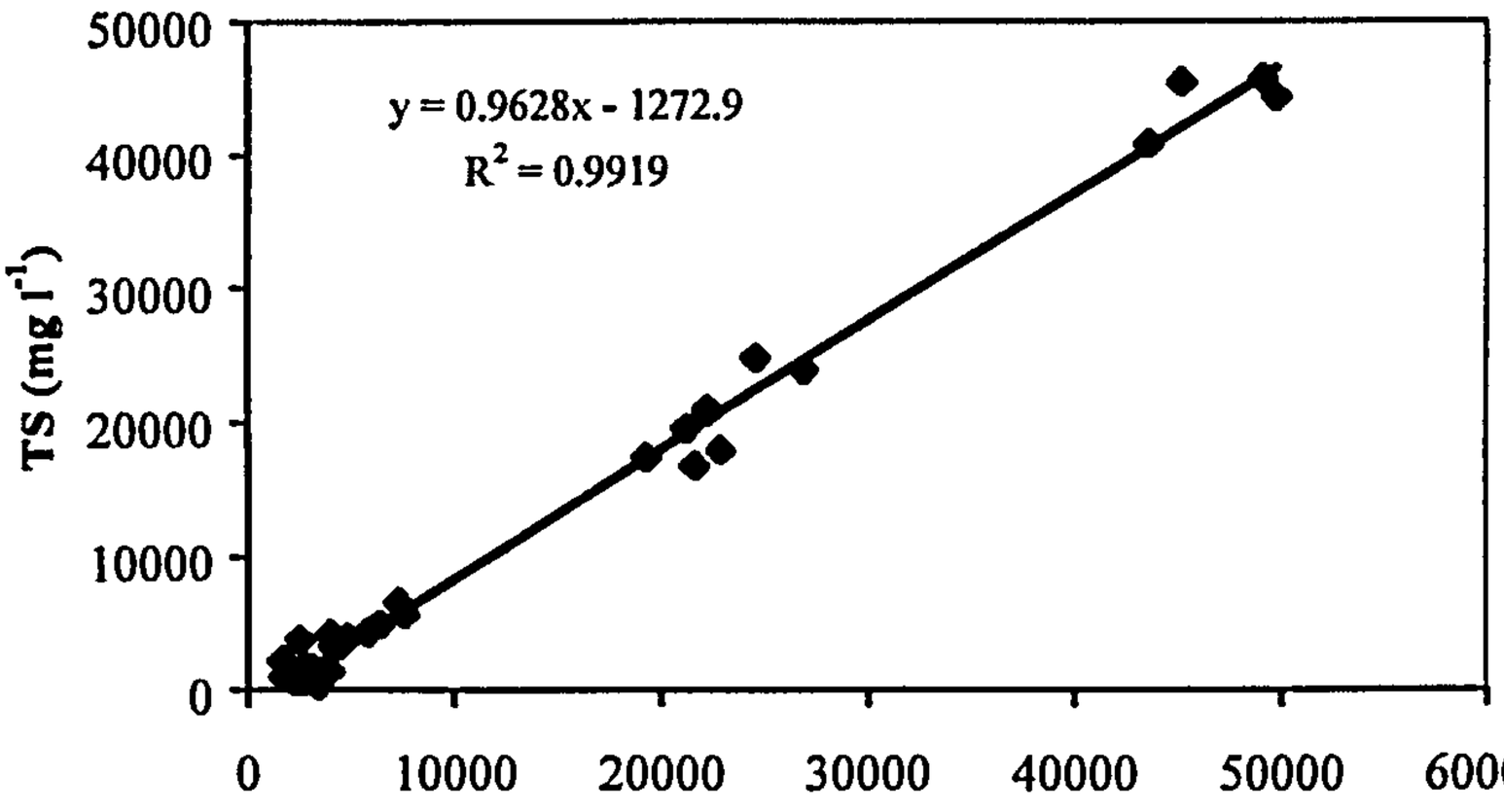


Figure 6.6. TS and SS concentration correlation with trace elements added.

6.2. ULTRASONICATION AS A PRE-TREATMENT METHOD

6.2.1. Experimental Procedure

The pre-treatment of aquaculture effluents using ultrasonication was examined under psychrophilic conditions. When sonication was applied, the aquaculture waste effluents were ultrasonicated for 15 minutes using a 120 W sonication bath operating at a frequency of 50 kHz. This represents a dose of 54 KJ g TS^{-1}, exceeding the optimum dose of 1.5 – 3.0 KJ g TS^{-1} recommended by Forster et al. (2000). The time period of 15 minutes was chosen because it corresponded to that previously documented to be ideal for activated sludge (King and Forster, 1990) and exceeded the 4 minutes previously reported to give appreciable changes by a considerable margin (Forster et al., 2000), while the sonication level (total energy input) was also higher than that previously shown to be effective (Forster et al., 2000). Temperature was not monitored during sonication.

The duration of the experiment was 202 days. The reactor was fed untreated aquaculture waste until day 48 when ultrasonication of the waste as a pre-treatment method commenced. A period of 48 days was considered adequate to establish performance with untreated waste because the digester had already been operated with the same feed for around 6 months before the start of the study. Ultrasonication of the feed stopped on day 155 and the digester continued to be fed with untreated waste until day 202. There was a pause in feeding between days 105 - 124 due to a holiday period. The biogas production was not measured during this period and no samples were taken for analysis. The period in which ultrasonication was employed was extended beyond the three retention times normally used to establish steady state to ensure the holiday period did not affect the performance. The final period of the study, in which no ultrasonication was employed, was not extended because it had become apparent that performance parameters such as

biogas production had stabilised and constraints such as equipment availability made it undesirable to continue further.

6.2.2. Effect of Ultrasound on the Digestion Process

Mixed liquor TS and SS were monitored throughout the study (Figure 6.7). The solids concentration was relatively stable until day 30 but rose by 20,000 mg l^{-1} between days 30 and 79. This accumulation of solids within the digester then declined between days 90 and 154. This decrease in solids may be attributed in part to a break in feeding from days 105 - 124. Although there was a slight increase in digester solids concentration, it remained relatively stable at 35,000 mg l^{-1} from day 154 until the end of the experiment on day 202.

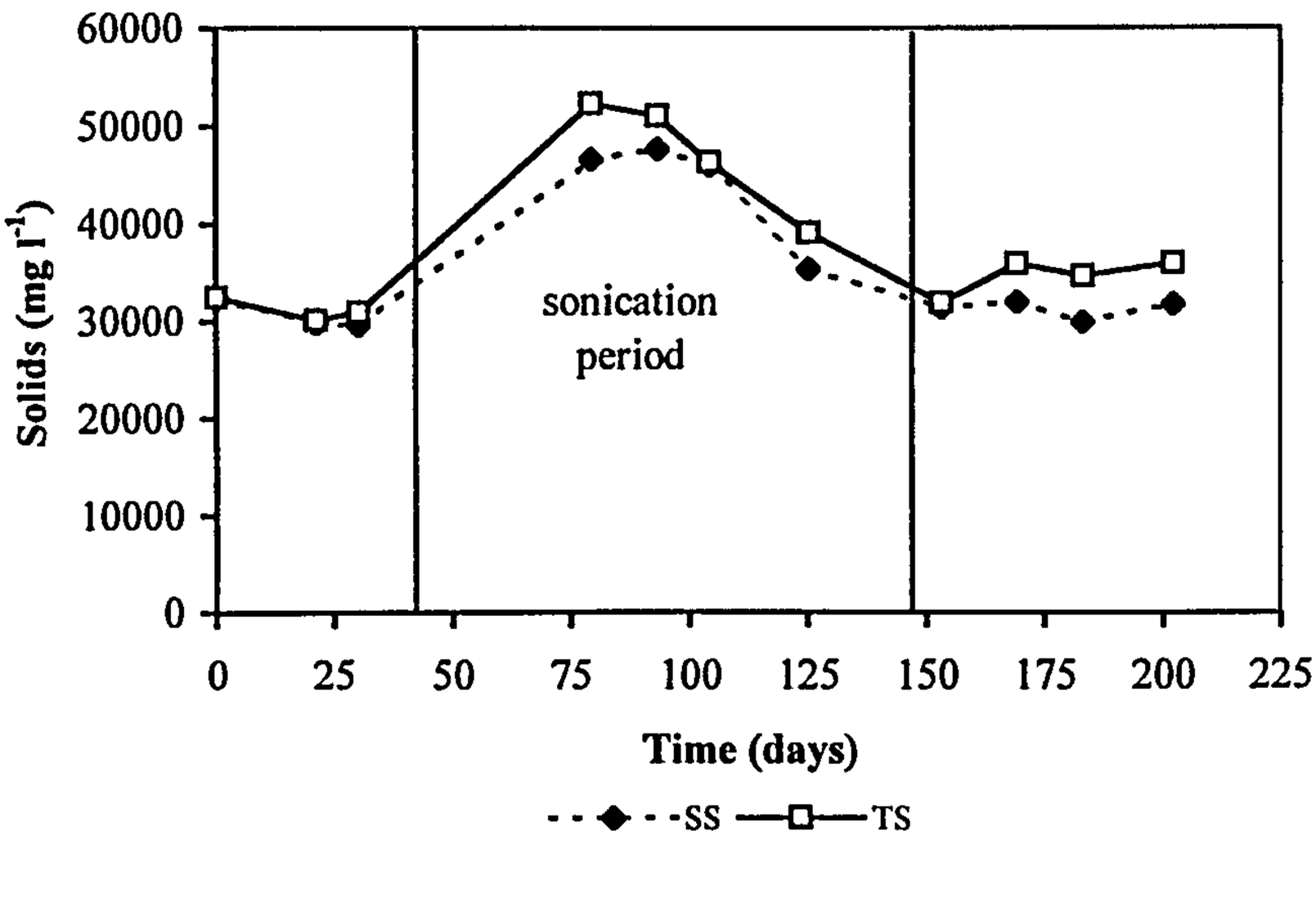


Figure 6.7. Anaerobic digester mixed liquor TS and SS concentrations throughout study.

Aquaculture waste feed for the digester had a COD concentration ranging between 7,000 and 10,000 mg l^{-1} (mean = 8663.4, SD = ± 1225.94, where $n = 25$). COD removal for the sonication period was *c.* 85% compared to 77% for the untreated waste periods (Figure

6.8). There was no statistical difference (one-way ANOVA) in reactor effluent soluble COD values between the two feeding regimes (where $n = 14$, $F_{1, 12} = 1.93$, $P < 0.05$) with typical levels of 120 mg l^{-1}. Similarly, BOD reduction was not noticeably enhanced by the use of ultrasonication as a pre-treatment method. For the entire period of digestion (days 0 - 202), a *c.* 90% reduction in waste BOD resulted in an average effluent BOD of 170 mg l^{-1}.

VFA concentrations, predominantly composed of acetic acid, were under 1,000 mg l^{-1} for the entire digestion period indicating a stabilised degradation process. The ultrasonication of the aquaculture waste did not have any noticeable effects in terms of VFA concentrations, which ranged from 65 - 530 mg l^{-1} during the pre-treatment trial. Alk values were also relatively constant throughout the study; from day 0 - 105 values for Alk ranged between 2,375 - 4,000 mg l^{-1} dropping to a range of 2,375 - 2,750 mg l^{-1} for the remainder of the study.

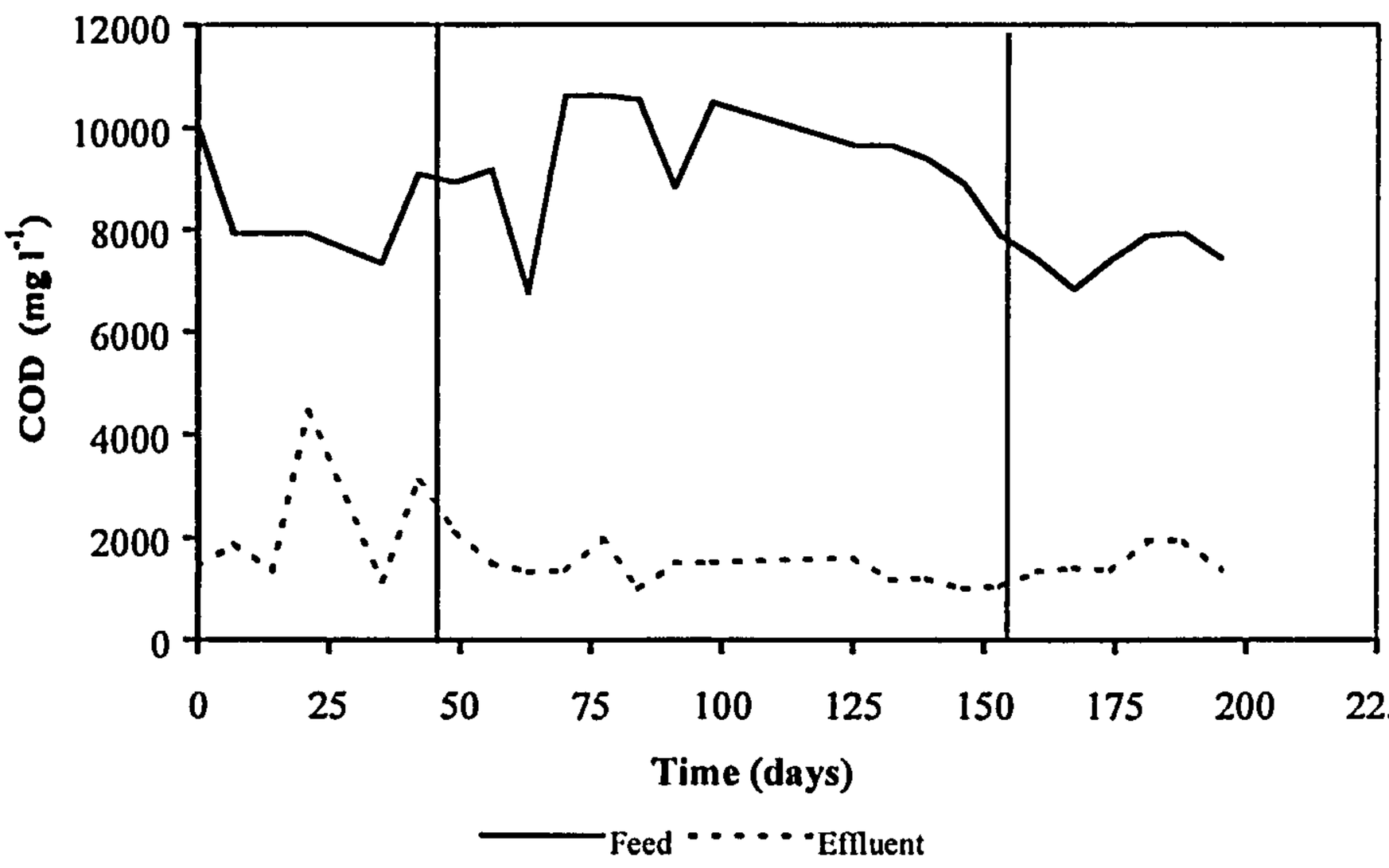


Figure 6.8. COD feed and effluent concentrations.

The reduction of reactive P was monitored throughout the study period. There was a noticeable increase in the removal of reactive P during the period of feed pre-treatment (Figure 6.9). Typical P effluent values ranged between 100 - 200 mg l^{-1} resulting from 77% and 88% removal efficiency for the untreated and sonicated feed respectively. Similarly, during the ultrasonication period, there was a substantial increase in the production of TAN (Figure 6.9). There was a 10 - 15% increase in TAN concentration with average effluent values of 220 ± 105 mg l^{-1}, presumably due to the release of nitrogenous compounds during the sonication of aquaculture waste.

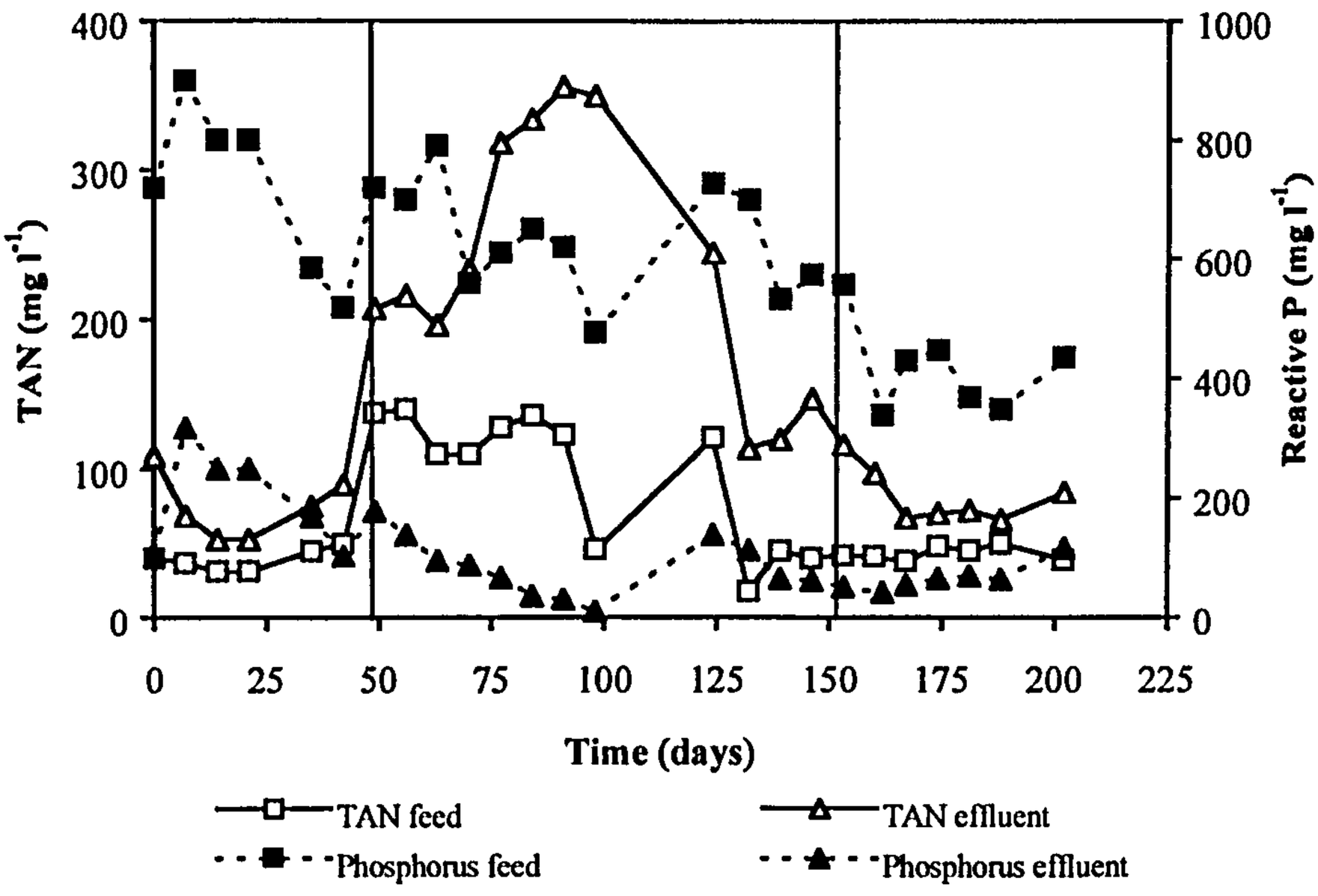


Figure 6.9. TAN concentrations of digester feed and effluent together with digester feed and effluent reactive P concentrations.

The main difference between the two feeding regimes was in biogas production. There was a greater generation of biogas during the sonication period with an average daily production of 0.35 ± 0.1 l day^{-1} (days 48 – 154) compared to 0.29 ± 0.09 l day^{-1} (days 0 – 47, 155 – 202) without pre-treatment (Figure 6.10). Between days 48 - 72, there was a

large decrease in the biogas production with *c.* 0.08 ± 0.02 l day^{-1} produced. It is unclear why biogas production declined, given the stability of the process in terms of VFA and Alk concentration. However, the decrease in biogas yield was in conjunction with an increase in mixed liquor SS. CH_4 content of biogas increased from 55% to 60 - 65% when the aquaculture waste was subjected to ultrasonication.

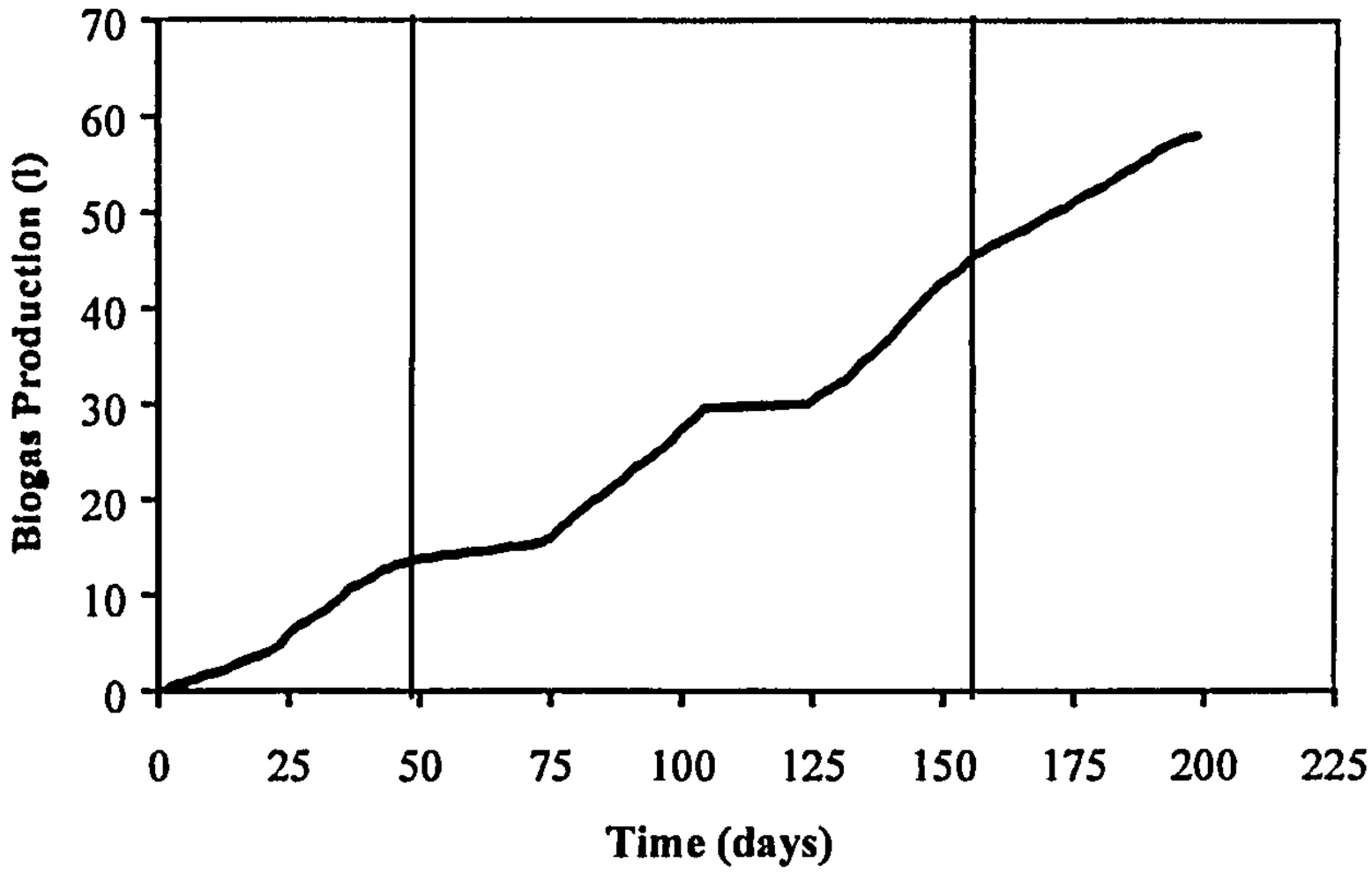


Figure 6.10. Cumulative biogas production for both untreated waste (days 0 - 48, and 155 - 202) and sonicated waste (days 49 - 154).

6.2.3. Discussion

Pre-treatment of the aquaculture waste by means of ultrasonication did appear to improve the performance of the psychrophilic digestion process. The stability of the digestion process was unaffected by the sonication of digester feed. If it is reasoned that the sonication of waste before digestion leads to a decrease in solid particle size, thus allowing for greater hydrolysis of waste material then it may also be considered that the rate of acidogenesis may increase with a subsequent increase in VFA concentrations. Wang et al. (1997) found that pre-treating digester feed led to an increase in VFA

concentrations, which sometimes could exceed inhibitory levels. However, VFA levels were found to be not greater than 530 mg l^{-1} during the ultrasonication period in the present study. In addition, the VFA:Alk ratio was found to be on average <0.1, which is a performance parameter indicative of stability as reported by Parkin and Owen (1986).

The disintegration of the waste during ultrasonication also increased the rate of total COD reduction in comparison to untreated waste during biodegradation. Tiehm et al. (1997) showed that pre-treatment of the sludge increased the soluble COD concentration. Similarly, Chiu et al. (1997) reported that waste activated sludge samples under a variety of pre-treatment methods such as alkaline solubilisation, and simultaneous ultrasonication and alkaline solubilisation released from 36% - 89% of total COD in the form of soluble COD. Wang et al. (1999) reasoned that the disintegration of solids allowed for the greater accessibility of hydrolytic bacterial enzymes to the intracellular organic substances. It would, therefore, be expected that this would result in a greater overall reduction of total COD.

Further evidence of the effect of ultrasonication on the digestion process can be seen through an examination of the TAN concentrations during degradation. The mechanical break up of waste particles increases the surface area allowing greater access for hydrolytic enzymes to release ammonia from proteins in the waste. Similarly, sonication reduced the reactive P concentration by a further 10%. This may be attributed to the break up of solid particulates thus releasing P from the solid matrix. This in turn may have allowed for greater utilisation by microorganisms or greater attachment of P to solid particulates due to an increase in particle surface area.

The biogas yield observed during the pre-treatment period was significantly greater than that in the untreated periods. There was a 20% increase in the daily biogas production

when aquaculture waste was ultrasonicated. This may be accounted for by the break up of particles by the ultrasonication of the raw aquaculture waste, allowing greater hydrolysis of organic matter and hence an increase in the conversion of organic material to biogas through methanogenesis. However, the start-up period of ultrasonication from days 48 - 72 had an extremely poor biogas production rate (*c.* 0.080 l day^{-1}). This may be expected if due to waste pre-treatment, a greater concentration of VFA was released, inhibiting the digestion process. However, VFA concentrations did not exceed 600 mg l^{-1} during this period (*c.* 200 mg l^{-1}). There was however an increase in the digester liquor solids content during this period which suggests sedimentation or a period of biomass growth within the reactor rather than digestion of organic material.

Tiehm et al. (1997) reported a greater increase in VS reduction (10%) at a HRT of 22 days with disintegrated raw sludge but no increased production in biogas when compared to the digestion of untreated sludge under similar conditions. This may be attributed perhaps to changes in the fermentation process and in the case of this study, a period of acclimatisation (days 48 - 76) may have been required to adjust to this change. If this period is removed from the data set, there is an even greater yield in biogas production (55% increase) when compared to the untreated feeding regime.

Statistical analysis (Mann-Whitney *U*-test) (Fowler et al., 1999) of the biogas data indicates that there is a significant difference (where $n = 179$, $P = 0.0121$, using degree of confidence of 95%) between the biogas yields for untreated and ultrasonicated aquaculture waste. Furthermore, calculation of the CH_4 yield per unit COD removed also shows an increase due to sonication of the aquaculture waste. Untreated aquaculture waste had a CH_4 yield of 0.06 in comparison with that of sonicated waste which was found to be 0.08 m^3 CH_4 kg COD_{rem}^{-1}.

6.3. CO-DIGESTION OF AQUACULTURE EFFLUENTS WITH CATTLE SLURRY

6.3.1. Experimental Procedure

Thermophilic anaerobic digestion of aquaculture effluent and cattle slurry at varying mixture ratios was studied with the view of enhancing the digestion process and examining the feasibility of aquaculture effluent co-digestion with cattle slurry. Three anaerobic reactors as described in *Section 3.3.1* operating at thermophilic temperatures and a HRT of 23.33 days were used for the co-digestion experiment. Mixed digester liquor from two 4 l anaerobic reactors, a thermophilic and mesophilic reactor, was combined and the liquor volume adjusted to 12 l using 2 l of water and 2 l of aquaculture waste effluent. The 12 l mixed liquor solution was then used for the start-up of three thermophilic reactors.

The cattle slurry was obtained from the housing area of a local dairy farm, stored in a plastic container premixed with aquaculture effluents and kept under refrigeration for the duration of the experiment. Two anaerobic digesters were fed a waste stream of aquaculture effluents to cattle slurry at a mixture ratio of 10:1 and 4:1 respectively. Mixture ratios were calculated on the basis of COD. As a control, aquaculture effluent was fed to a thermophilic digester without the addition of cattle slurry. Approximate values for cattle slurry and aquaculture effluents used for digester feeding are shown in Table 6.4. All reactors were initially fed aquaculture waste only (days 0 - 39). The reactors fed mixture ratios 4:1 (25% cattle slurry) and 10:1 (10% cattle slurry) were designated T1 and T2 respectively. The control reactor, fed aquaculture effluent only, was designated T3. The duration of the experiment was 132 days.

Table 6.4. Digester Cattle slurry and Aquaculture effluent feed concentrations (Mean ± STD).

Parameter	Cattle Slurry (mg l^{-1})	Aquaculture Effluents (mg l^{-1})
COD	22,057.7 ± 13,912.8	8,827.7 ± 1,174.8
DRP	1,447.4 ± 862.4	221.3 ± 201.9
TAN	249.3 ± 187.4	40.4 ± 9.5
TS	6,939.7 ± 6,630.1	3,7538.2 ± 658.9
SS	5,237.2 ± 2,761.5	2,212.7 ± 535.2

6.3.2. Effect of Co-digestion on the Digestion Process

The waste feed mixtures for the reactors T1, T2 and T3 had mean COD concentrations of 9,933 ± 1,809, 10,434 ± 1,817 and 9,031 ± 1,286 mg l^{-1} respectively. COD removal for the period of cattle slurry addition (days 40 - 132) was 75, 80, and 77% for T1, T2 and T3 respectively (Figure 6.11). There was slight increase in the soluble COD concentration for digesters fed with the cattle slurry mixture in comparison with the control reactor. Mean values and SD (n = 11) for effluent soluble COD were 350 ± 140.1, 281 ± 83.6 and 254 ± 89.4 mg l^{-1} for T1, T2 and T3 respectively. Statistical analysis (one-way, ANOVA) indicated that the BOD reduction was similar for all three reactors with a percentage reduction of *c.* 93% (where $n = 18$, $F_{2, 15} = 1.02$, $P < 0.05$,).

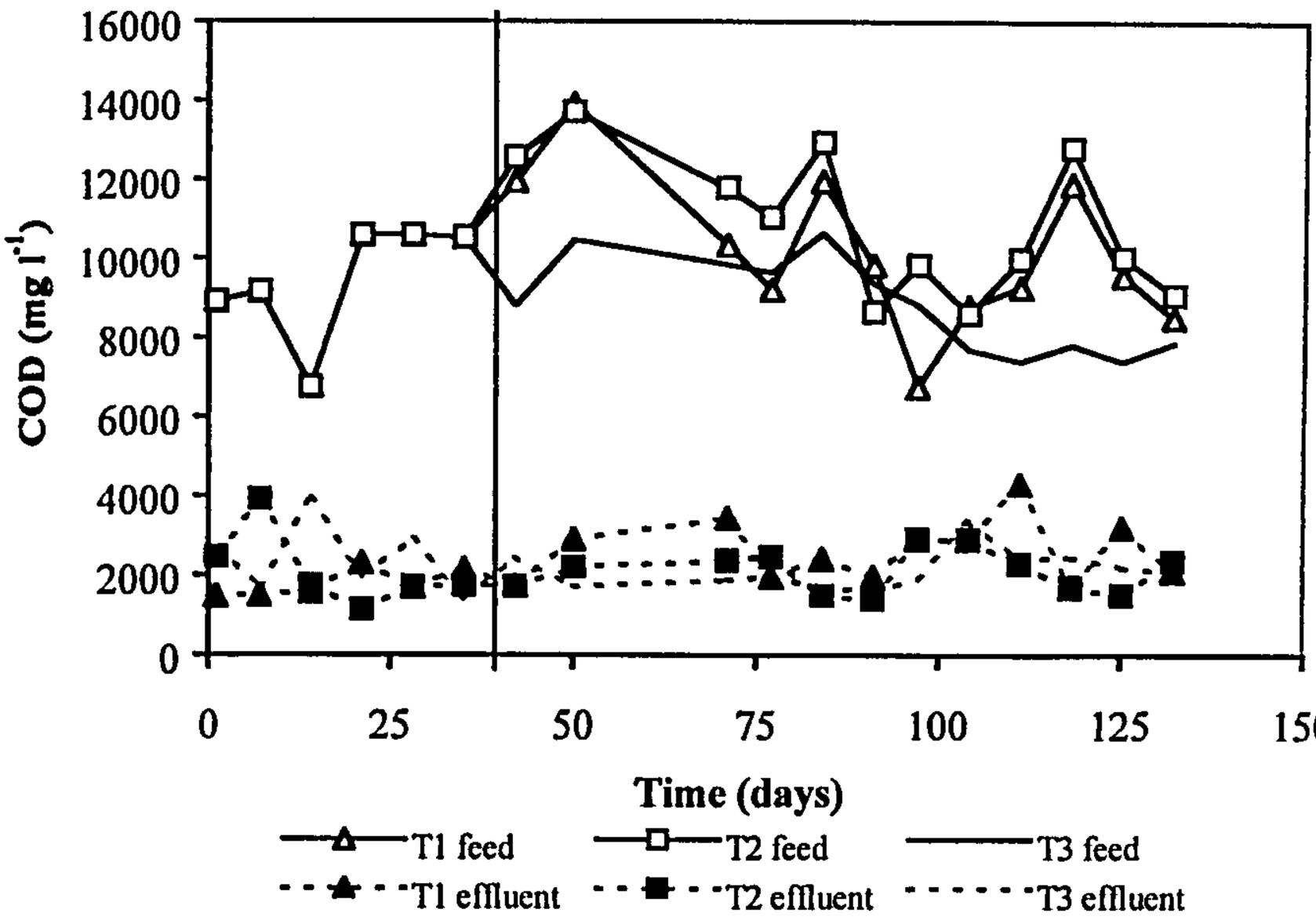


Figure 6.11. COD of feed and effluent for reactors T1, T2 and T3.

The accumulation and removal of TAN and P were monitored periodically throughout the experiment. Mean TAN feed concentrations were 138 ± 89, 107 ± 62 and 70 ± 48 mg l^{-1} for T1, T2 and T3 respectively indicating a greater concentration of TAN in the cattle slurry in comparison with aquaculture effluents. Effluent TAN concentrations increased by 51, 88, and 147% for T1, T2 and T3 respectively indicating a greater release of TAN from the feed stream containing the greater proportion of aquaculture effluents. In contrast, the reduction of reactive P was greatest for the digester feed containing cattle slurry, 76 and 69% respectively for T1 and T2. P reduction for the control reactor, T3, was 46%.

Digester mixed liquor TS and SS concentrations for all three reactors were initially equal at day 0 (Figure 6.12). A sharp rise in the TS and SS concentrations was then observed. Between days 0 – 55, there was a rise in TS and SS in reactor T1 from 22,680 and 14,440 mg l^{-1} to 47,810 and 43,870 mg l^{-1} respectively. There was also a rise in TS and SS in T2 between days 0 – 55 to 36,050 and 34,895 mg l^{-1}. Similarly, TS and SS in T3 rose to

29,410 and 27,240 mg l^{-1}. Digester liquor TS and SS in T1 then levelled off and there was a slight decrease between days 104 – 132 to 44,510 and 36,270 mg l^{-1} respectively. Reactor T2 TS and SS concentrations also began to decrease after day 104 until the end of the experiment to 30,180 and 19,640 mg l^{-1} respectively. This suggests active digestion was taking place within both T1 and T2 and rather than accumulation of solid material. T3 mixed liquor solids remained relatively constant from day 55 until day 120, when there was a sharp increase in TS (45,520 mg l^{-1}) followed by a decrease to 35,910 mg l^{-1} on day 132.

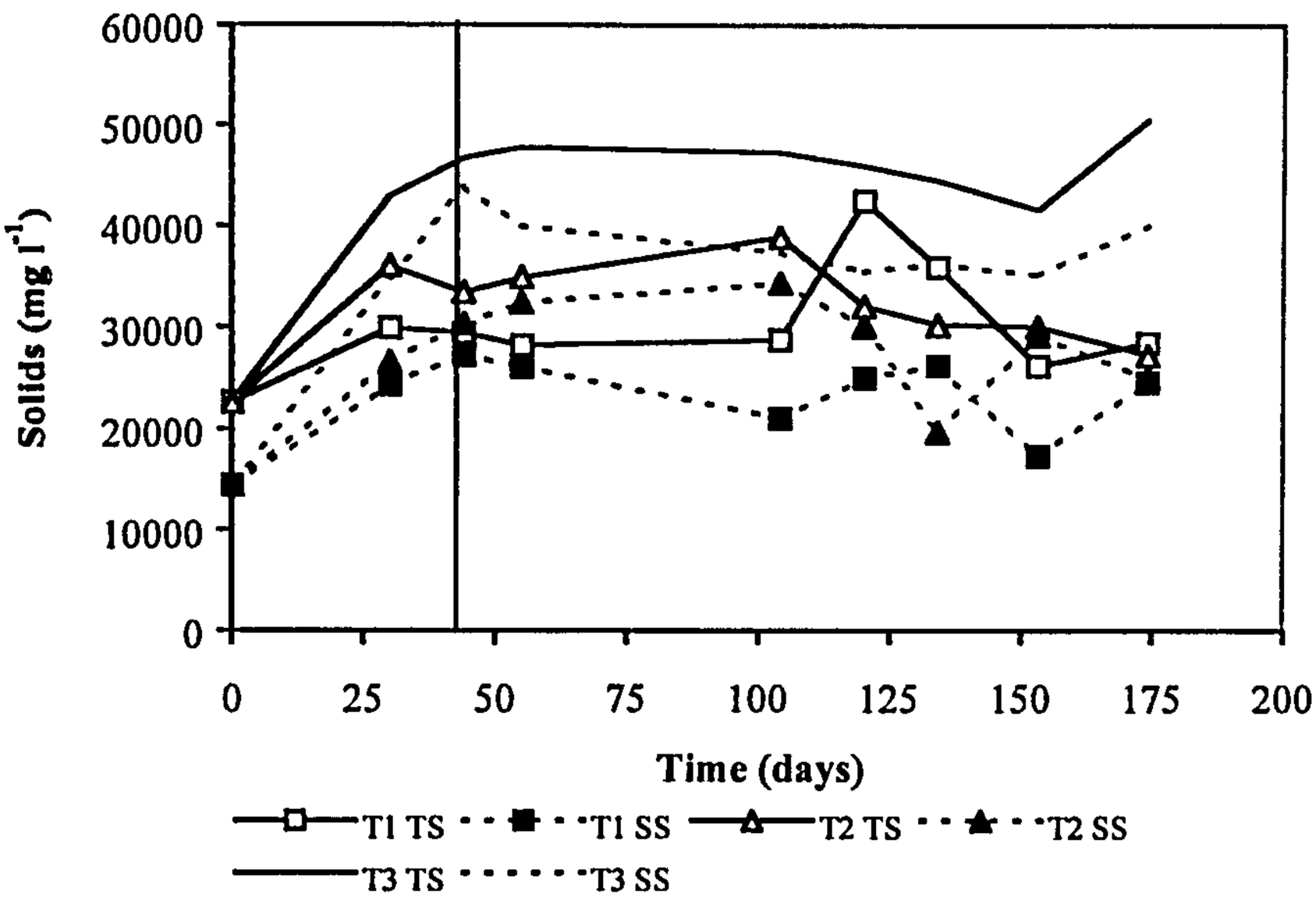


Figure 6.12. Digester liquor TS and SS concentrations.

Mean anaerobic digester feed and effluent solid concentrations were relatively constant throughout the experiment (Table 6.5). However, TS and SS reduction in all three reactors was very poor. SS reduction for T1, T2 and T3 was 33, 38 and 24% respectively.

Table 6.5. Feed and effluent TS and SS concentrations (Mean ± SD).

		T1 (mg l^{-1})	T2 (mg l^{-1})	T3 (mg l^{-1})
Feed	**TS**	4407 ± 1093	4223 ± 699	3932 ± 522
	SS	2566 ± 581	2289 ± 480	2223 ± 511
Effluent	**TS**	3689 ± 849	3239 ± 739	3122 ± 556
	SS	1691 ± 421	1419 ± 418	1693 ± 472

VFA concentrations for all three reactors were below 700 mg l^{-1} throughout the study. The predominant fatty acids were acetic and propionic acid, indicating a stable process. VFA peaked during the start up period from days 0 – 28 at concentrations of 548, 670 and 490 mg l^{-1} for T1, T2 and T3 respectively. Similarly, VFA peaked after the Christmas vacation period between days 77 - 96 when feeding recommenced with values of 525, 237 and 275 mg l^{-1} for T1, T2 and T3 respectively. The addition of cattle slurry to the digester feed did not have an adverse effect on the performance of the digester in terms of VFA degradation; Alk throughout the study was typically 2,000 mg $CaCO_3$ l^{-1} for all three reactors. The Alk reduced around day 98 due to the failure of the heating system. For all three reactors the Alk was reduced to *c.* 1,000 – 1,250 mg $CaCO_3$ l^{-1} but returned to typical concentrations by day 104 with the addition of sodium bicarbonate.

Initially, all three digesters were fed aquaculture waste effluents only (days 0 – 39) and it would therefore be expected that biogas production would be comparable for T1, T2 and T3. This did occur between days 0 - 14, but there was a greater production of biogas by reactor T1 from day 14 to the end of the experiment (Figure 6.13). This result may be attributed in part to the addition of cattle slurry from day 40 onwards. Prior to this period when digester feed was similar for all reactors, the greater yield of biogas must result from environmental conditions within the digester. Temperature fluctuations occurred in reactors T2 and T3 between days 0 – 14 and 0 – 44 respectively. Due to the failure of the

heating apparatus, temperature was 5 - 10°C below thermophilic conditions during these periods. This would have resulted in the poor performance of thermophilic bacteria in terms of substrate degradation and methanogenesis. The addition of cattle slurry to the digesters resulted in a decrease in the rate of biogas production. Mean values for daily biogas production were 0.55 ± 0.1 and 0.31 ± 0.08 l for T1 and T2 prior to cattle slurry addition and 0.39 ± 0.07 and 0.29 ± 0.06 l respectively from days 40 – 132. Daily biogas production from T3 was relatively constant throughout the experiment, with a mean value of 0.22 l.

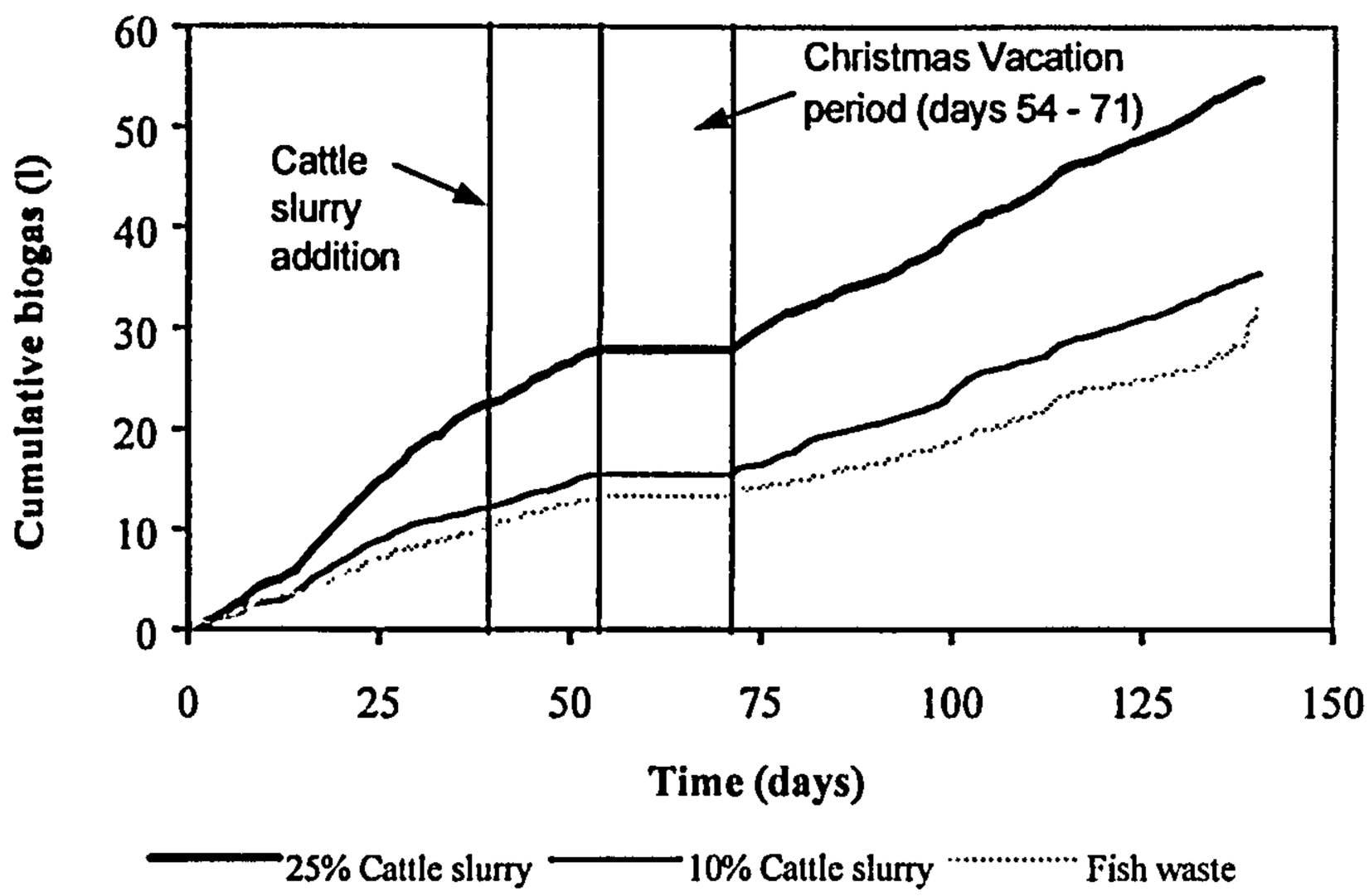


Figure 6.13. Cumulative biogas production for reactors T1, T2 and T3 for the duration of the experiment, days 0 – 140.

The % CH_4 content was slightly greater for the control reactor, T3, fed only aquaculture effluents and from days 0 – 98 was 54, 56 and 58% for reactors T1, T2 and T3 respectively (Figure 6.14). Temperature fluctuations resulting from the failure of the heating system resulted in low CH_4 content after day 98. CH_4 content values were typically as low as 39, 37 and 43% for T1, T2 and T3 respectively. The CH_4 content of

biogas from all three reactors returned to typical levels on day 119, with values in the range of 51 - 55% by day 133.

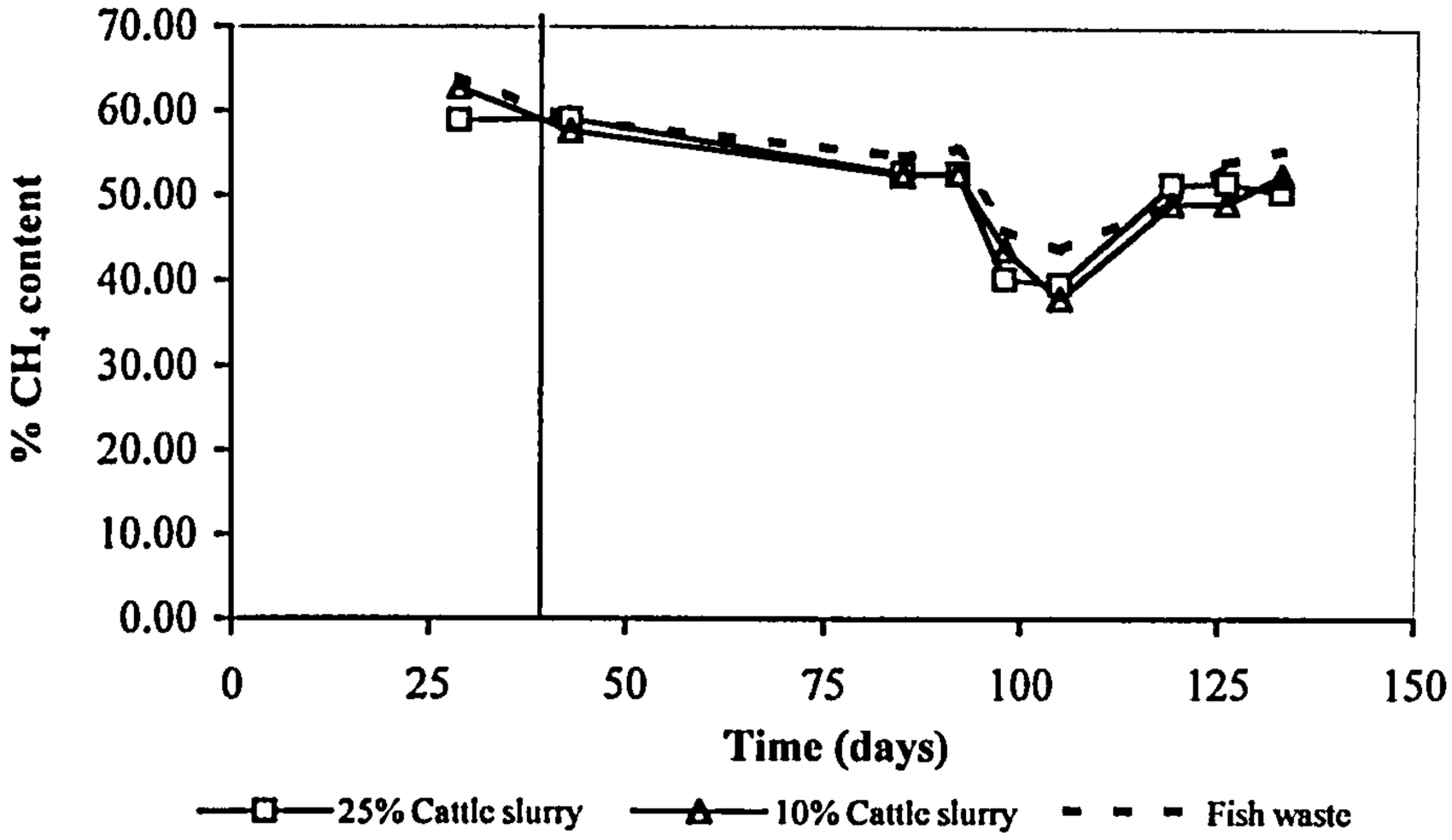


Figure 6.14. Percentage CH_4 content of biogas from digesters T1, T2 and T3.

6.3.3. Discussion

Due to the failure of the heating system, temperature fluctuations were evident at the start of the study. Temperatures were in the range 45 - 49°C for all three reactors at start-up. Temperature within digester T1 was stabilised at 55°C on day 5 whereas T2 and T3 were stabilised on day 16. The impact on digester performance as a result of this temperature difference was evident from an examination of cumulative biogas yield (Figure 6.13). It can be seen that there was *c.* 45 - 50% increase in biogas production for T1 compared to T2 and T3 respectively during the period 0 – 16 days.

The effect of temperature on the digestion process and hence CH_4 production has been investigated by many researchers (van Velson and Lettinga, 1979; Van Lier et al., 1990;

1992; 1997; Nozhevnikova et al., 1999; Masse and Masse, 2001). Van Lier et al. (1992) demonstrated the exponential increase in CH_4 production rate with an increase in anaerobic digestion operating temperature from 55°C to 64°C. van Velson and Lettinga (1979) reported the rapid decrease in biogas production during the anaerobic digestion of piggery manure when temperature was reduced from 30°C to 20°C for one day.

Even with the stabilisation of all reactors at 55°C, it was still evident that there was a greater biogas yield from reactor T1. This may be explained in part to a greater accumulation of solid particulate in digesters T2 and T3 (Figure 6.12) suggesting a greater degradation of organic material in T1, thus greater biogas production. Furthermore, this would concur with a study by Van Lier et al. (1990) which reported an exponential decrease in CH_4 production rate due to bacterial decay when temperature was increased from 36°C to > 45°C.

Temperatures for T2 and T3 were in the range 45 - 49°C, which are close to the values reported to be too low for thermophiles and too high for mesophiles (Henze and Harremoes, 1983). It therefore may be reasoned that the lower biogas yields for T2 and T3 were due to low bacterial activity, demonstrated also by the accumulation of solid material within the reactors, as a result of the decay of mesophilic bacteria (digester contained a mixture of mesophilic and thermophilic bacteria from start-up, *Section 6.3.1*) and slow growth rate of thermophilic bacteria. Reactor T2 had a slightly greater biogas production (*c.* 10%) in comparison with T3 between days 16 and 27 after temperature stabilisation. This may be explained by a concurrent increase in the reduction of COD within T2 during this period. Between days 27 – 40 the biogas production for both T2 and T3 was similar at 225 and 221 ml day^{-1}, but still approximately 50% below the daily yield of T1. Van Lier et al. (1992) reported an acclimatisation period of 1 - 2 weeks for stable

thermophilic methanogenesis to occur after transition from mesophilic temperatures. It is therefore not clear why the biogas yield for T2 and T3 was lower than that of T1, but it is postulated this may be due to the greater need for acclimatisation of mesophilic bacteria to thermophilic temperatures.

The failure of the heating system again between days 98 - 99 also had an adverse impact on the performance of the digesters. Although the biogas production for all three reactors in the present study was not noticeably hindered, the composition of biogas in terms of CH_4 content was reduced by *c.* 10% for each digester. Anaerobic bacteria operating at thermophilic temperatures are known to be more susceptible to environmental change (Ahn and Forster, 2000), in particular the methanogenic bacteria which are known to be more sensitive than other anaerobic trophic groups (McCarty, 1964; Van Lier et al, 1990). It would therefore be expected to find an increase in VFA concentration in digester effluents.

The consistently low VFA concentrations in all three reactors may be explained by the time of sampling of VFA from the effluent. Sampling of VFA was performed on days 97 and 104 while temperature fluctuation occurred on days 98 – 99. It is feasible that the digestion process destabilised within this period resulting in low VFA concentrations observed on the days of sampling. Evidence of increased VFA levels may be seen from an examination of Alk levels in the reactors. During the period of temperature decrease, Alk decreased from *c.* 2,750 to 1,100 mg $CaCO_3$ l^{-1} within all three digesters. It is assumed that this was due to the increase in VFA concentration and also the increase in aqueous CO_2 as a result of the increase in CO_2 solubility with decreasing temperature.

van Velson and Lettinga (1979) reported the recovery of stable methanogenesis after shock temperature fluctuations within 16 days. Recovery of CH_4 in this study took 21

days (from day 99 – 119) which is close to that reported by van Velson and Lettinga (1979), albeit with temperature fluctuations from 20 - 40°C in their study.

The introduction of cattle slurry to reactors T1 and T2 on day 40 had no noticeable adverse impact on digester stability in relation to VFA and Alk. Ammonia toxicity has been reported in the digestion of cattle manure (Hashimoto, 1986; Angelidaki and Ahring, 1993) and the inhibitory effect of unionised ammonia (NH_3) on methanogenesis is well documented (van Velson, 1979; de Baere et al., 1984; Koster and Lettinga, 1984; Webb and Hawkes, 1985; Heinrichs et al., 1990; Hansen et al., 1998). Ammonia inhibition is pH and temperature dependent due to the equilibrium between the ammonium ion (NH_4^+) and NH_3. The latter form is increasingly present at elevated pH, and acetoclastic methanogens are sensitive to its presence (Magbanua et al., 2001). An increase in pH or temperature will cause a shift in the equilibrium resulting in an increase in the concentration of NH_3.

The pH in all three reactors was relatively constant and ranged between 7.0 and 7.5 with mean digester effluent TAN concentrations of 207 ± 148.5, 200 ± 156.7 and 172 ± 81.2 mg l^{-1} for T1, T2 and T3 respectively. The proportion of NH_3 present in equilibrium with NH_4^+ may be estimated using the Henderson Hasselbach equation (Heinrichs et al., 1989):

$$[UAN] = \frac{[TAN]}{1 + 10^{(pKa - pH)}} \quad \text{..... (Equation 6.1)}$$

Where: $[UAN]$ = concentration of unionised ammonia;

[TAN] = concentration of total ammonia nitrogen;

pKa = the dissociation constant for NH_4^+ which depends on temperature and is equal to 8.41 at 55°C (Borja et al., 1996).

From the above equation, NH_3 concentration for reactors T1, T2 and T3 were calculated at 29.1, 23.2 and 20.0 mg NH_3 l^{-1} respectively. For unadapted methanogenic cultures, ammonia inhibition due to NH_3 has been reported to occur at concentrations ranging from 80 – 250 mg NH_3 l^{-1} (Callaghan et al., 1999). It may therefore be assumed that no inhibitory effect associated with ammonia occurred in this study.

It was observed that the concentration of feed TAN was greater for T1 and T2 and it may be expected that there would therefore be a greater increase in TAN effluent concentrations for the same reactors. However, the greater release of TAN in T3 may be explained by the greater presence of organic or biologically bound N in the aquaculture waste in comparison with cattle slurry, which would be undetected by the soluble ammonia nitrogen test. Organic N in the form of proteins, for example, would be degraded during the digestion process.

There was also an increase in the concentration of reactive P fed to the digesters relative to the proportion of cattle slurry added. Mean effluent reactive P concentrations were reasonably similar at 131 ± 96.7, 103 ± 51.9 and 146 ± 51.2 mg l^{-1} for T1, T2 and T3 respectively. As with N there may also be a greater proportion of P biologically bound in aquaculture effluents and consequently will not be detected during the analysis of reactive P. This may explain the increase in reduction of the P for T1 and T2 in comparison with T3. The removal of reactive P by reactor T3 (46%) in comparison to the previous experiments at thermophilic temperatures was poor (Table 6.2). It is possible that temperature fluctuations resulted in the decay of microorganisms and hence the release of constituents, including P, to the digester supernatant. This could explain the lower percentage removal of reactive P for reactor T3.

The best indicator regarding the effect of cattle slurry and aquaculture waste co-digestion on the performance of the digestion process was the impact on the biogas production. At first, it may appear that co-digestion had a strong inhibitory effect on biogas production. However, when the daily biogas productions of all three reactors are compared, this reduction is not as large. The mean daily biogas production for all three reactors when stabilised between days 27 - 40 was 0.54 ± 0.12, 0.23 ± 0.1 and 0.22 ± 0.08 l for T1, T2 and T3 respectively. The introduction of cattle slurry to the reactors T1 and T2 on day 40 saw this daily yield reduced to 0.36 ± 0.13 and 0.22 ± 0.09 l for T1 and T2 respectively. However, there was also a reduction in biogas yield from T3 by *c.* 10%, similar to that of T2. This suggests there was a general reduction in biogas yield for all three reactors and this may not solely be attributed to the use of cattle slurry in the feed stream. Taking this into consideration, it may be assumed that the cattle slurry reduced the biogas yield in T1 by *c.* 24% (when compared with biogas yield of T1 before introduction of cattle slurry) and not 34% as first appears.

Due to the initial temperature problems and acclimatisation of bacteria to cattle slurry for days 0 - 54, a greater understanding of the effects of co-digestion may be obtained by examining the biogas yield following the interruption of feeding during a holiday period (days 71 – 132). With the exception of days 98 - 99, which appeared to affect biogas composition and not yield, there were no problems associated with performance. Mean biogas yields from days 71 - 132 were 0.39 ± 0.11, 0.29 ± 0.07 and 0.25 ± 0.1 ml day^{-1} for T1, T2 and T3 respectively.

The COD reduction for the three reactors was similar at 75, 80 and 77% respectively. Although VS were not measured, the increase in biogas yield indicates the greater biodegradability and increased VS content of cattle slurry when compared to aquaculture effluents. However, it is difficult to evaluate the true influence of cattle slurry on the

digestion of aquaculture effluents due to the temperature problems during the study. Although no inhibition of digestion was observed in comparison with the control reactor, T3, CH_4 yields per kg of COD removed were low in comparison with those from previous experiments with aquaculture waste only (Table 6.2). CH_4 yields during this study were 0.14, 0.10 and 0.08 m^3 CH_4 kg COD_{rem} for T1, T2 and T3 respectively.

6.4. POST-TREATMENT OF ANAEROBICALLY DIGESTED AQUACULTURE EFFLUENTS

6.4.1. Experimental Procedure

The duration of the experiment was 146 days. An aerobic biofiltration unit (*Section 3.4.1*) was initially operated with total recycle of anaerobically digested effluents in order to encourage the growth of a biofilm (*c.* 22 days). The fixed film biofiltration unit was then fed an organic loading rate of 0.53 kg COD m^{-3} d^{-1} from days 0 - 133 and 1.04 kg COD m^{-3} d^{-1} for the remaining period of the study. Feed and effluent samples were analysed as described in *Section 3.3.4*.

6.4.2. Biofiltration of Anaerobic Effluents

Mean effluent TS concentration at a loading rate of 0.53 kg COD m^{-3} d^{-1} was 4,543 ± 556 mg l^{-1}, a reduction of *c.* 32% (Figure 6.15). There was a greater reduction in SS due to attachment to the media bed with mean effluent concentrations of 420 ± 214 mg l^{-1}. The increase in the organic loading from days 134 - 146 resulted in a slightly greater TS percentage reduction (35%), with mean effluent concentration was by far greater at 748 ± 75 mg l^{-1}. The increase in loading rate also had an impact on SS concentrations with percentage removal rate decreasing from 85 to 79%.

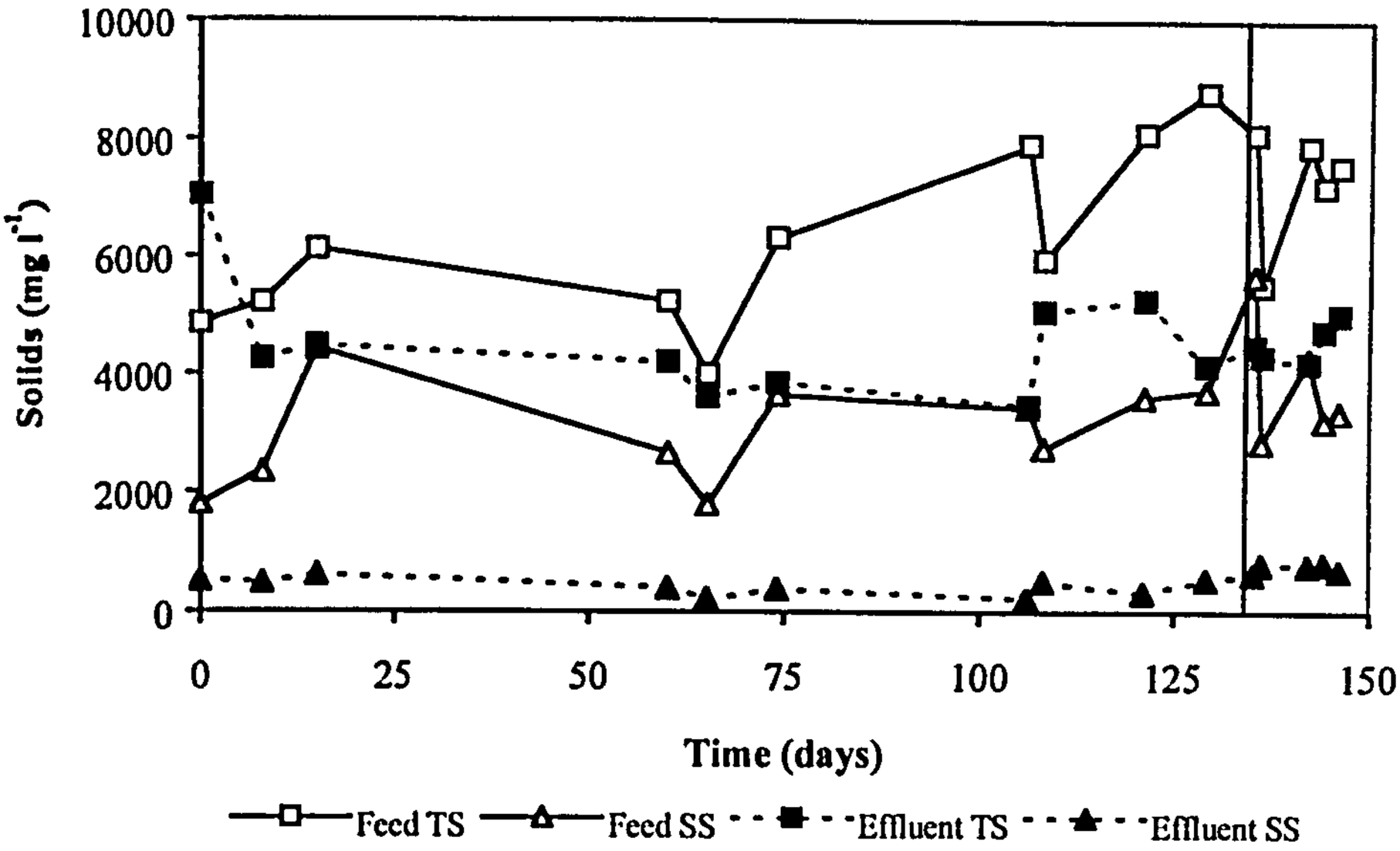


Figure 6.15. Feed and effluent TS and SS concentrations for the duration of the study.

There was a similar reduction in COD of 81 and 83% for both organic loading rates of 0.53 and 1.04 kg COD m^{-3} d^{-1} respectively (Figure 6.16). The slight increase in carbonaceous oxidation for the higher loading rate was due to the initial period of biofilm formation at the start of the study. The BOD was reduced from mean values of 310 ± 130 to 41 ± 19 mg l^{-1} and 338 ± 84 to 62 ± 19 mg l^{-1} for the low and high organic loading rates respectively. Percentage BOD removal was high throughout the study (Figure 6.17).

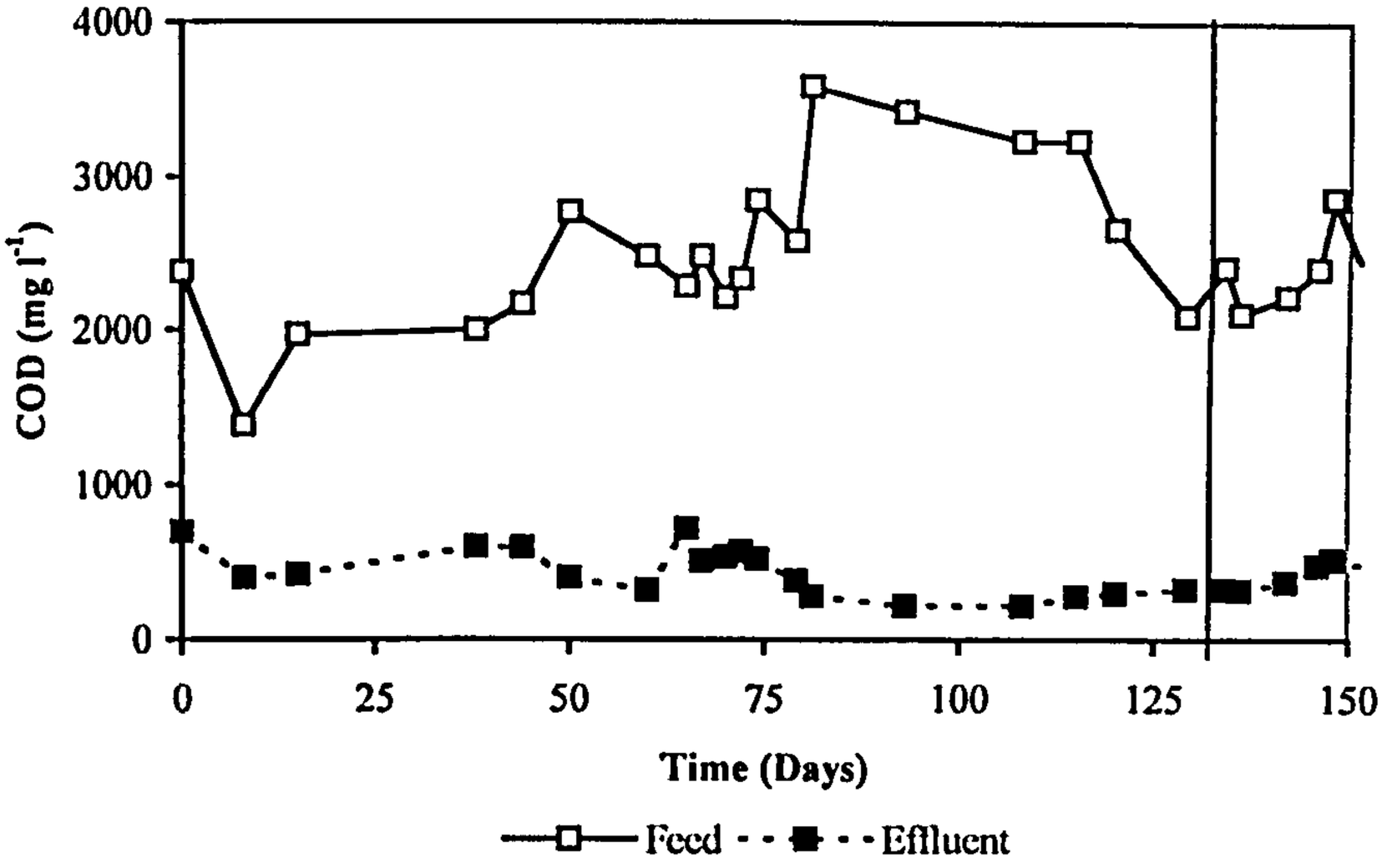


Figure 6.16. Feed and effluent COD concentrations for the duration of the study.

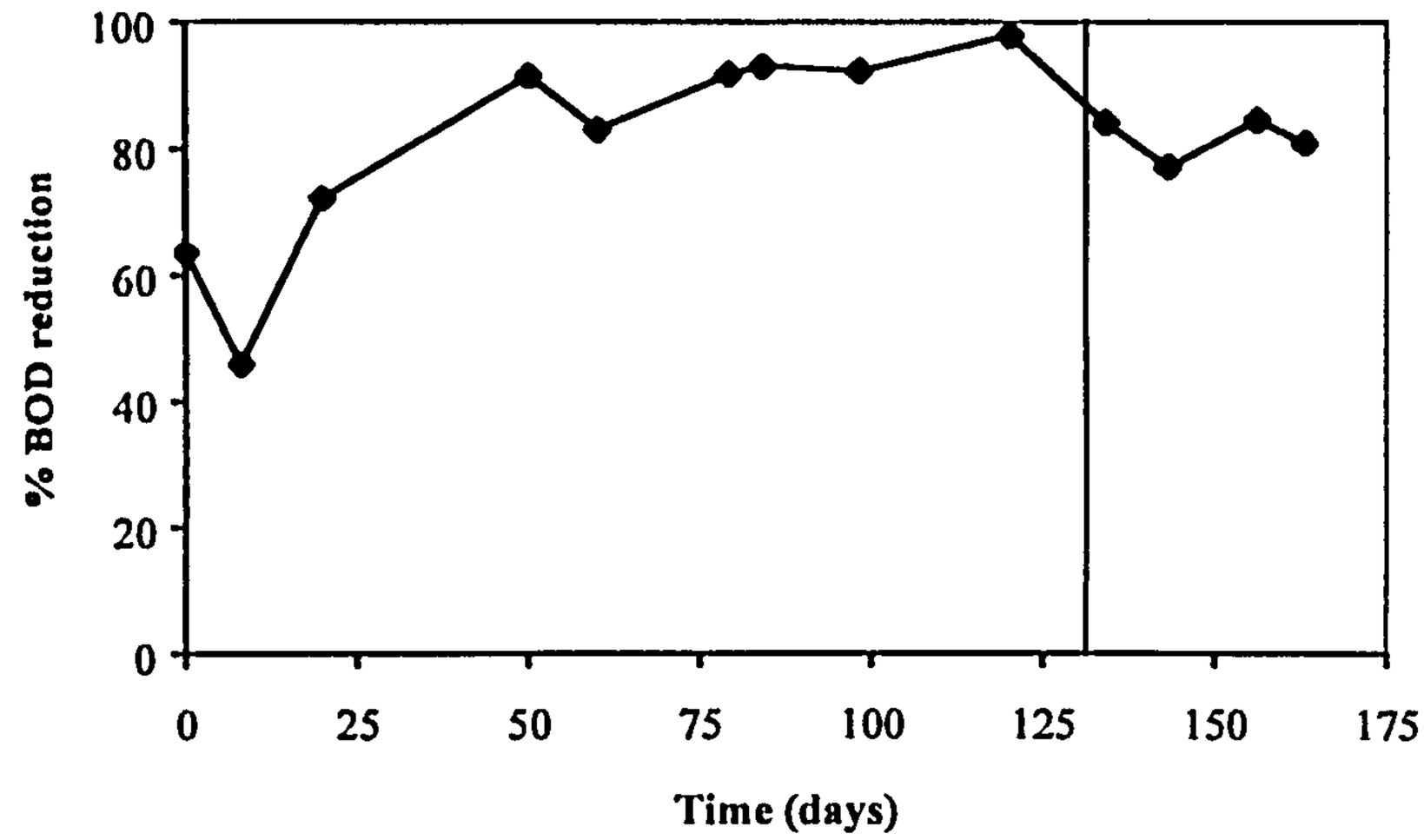


Figure 6.17. Percentage BOD removal of biofiltration unit for the duration of the study.

The nitrification of ammonia to nitrate was similar for both loading rates (Figures 6.18, 6.19 and 6.20). A period of acclimatisation/establishment of nitrifying bacteria was required. This can be seen at days 0 – 18 with mean effluent TAN concentrations of *c.* 30 ± 11.65 mg NH_4-N l^{-1}. After this initial period of growth, effluent TAN concentrations were typically less than 1 mg NH_4-N l^{-1} with mean concentration of 2.3 ± 3.51 mg NH_4-N

l^{-1} at a loading rate of 0.53 kg COD m^{-3} d^{-1}. There was a concurrent increase in NO_3-N with mean concentrations of 29.16 ± 12.02 mg l^{-1} after day 48. The increase in loading rate did not affect the nitrification performance of the biofilter. Mean effluent concentrations (inclusive of start-up period) at a loading rate of 1.04 kg COD m^{-3} d^{-1} were 0.44 ± 0.1, 0.09 ± 0.08 and 27.5 ± 13.5 mg l^{-1} for NH_4-N, NO_2-N and NO_3-N respectively. There was also was a reduction of *c.* 57 and 37% in reactive P concentrations for the low and high loading rate respectively.

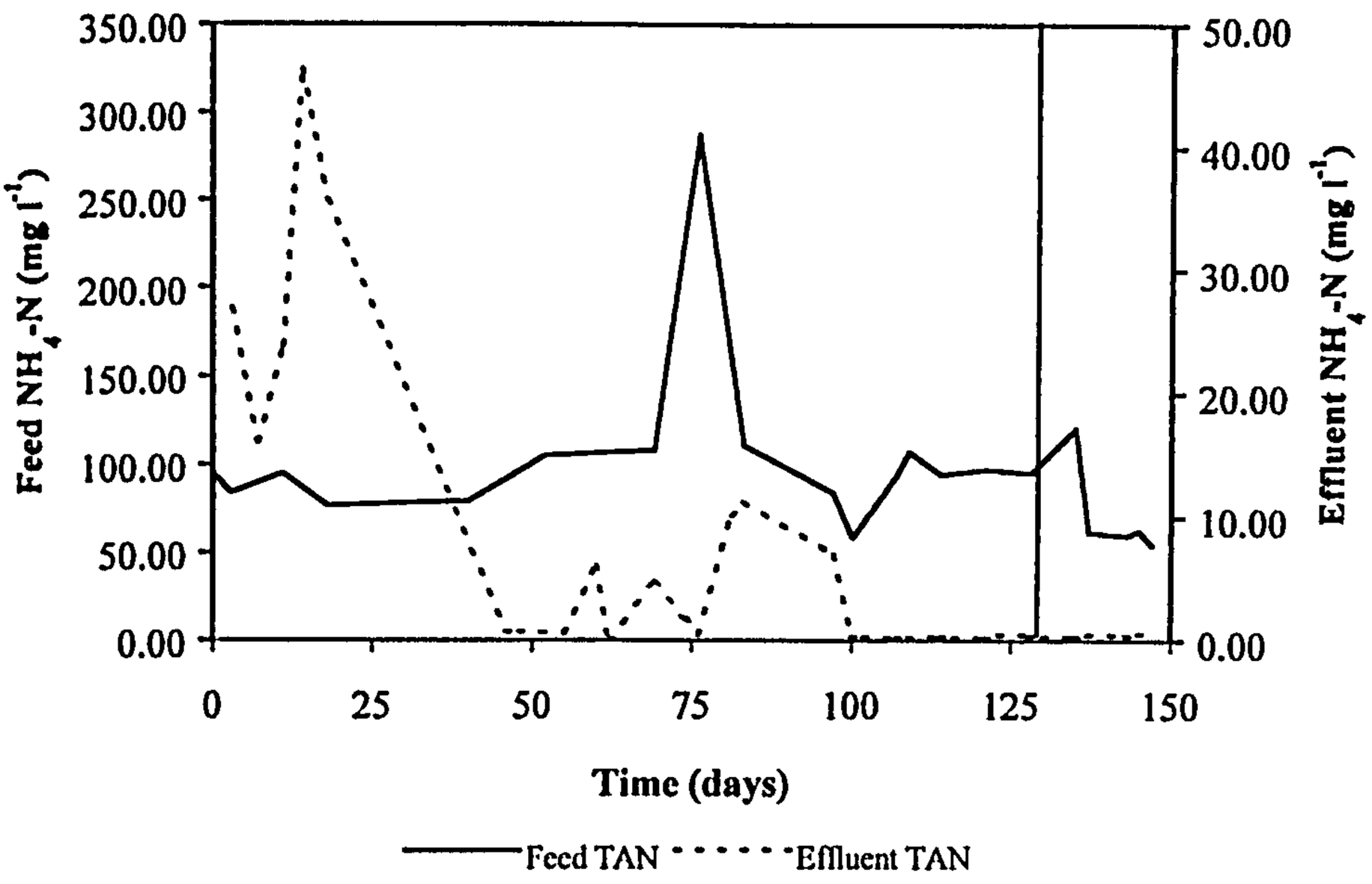


Figure 6.18. Feed and effluent ammonia nitrogen concentration for the duration of the study.

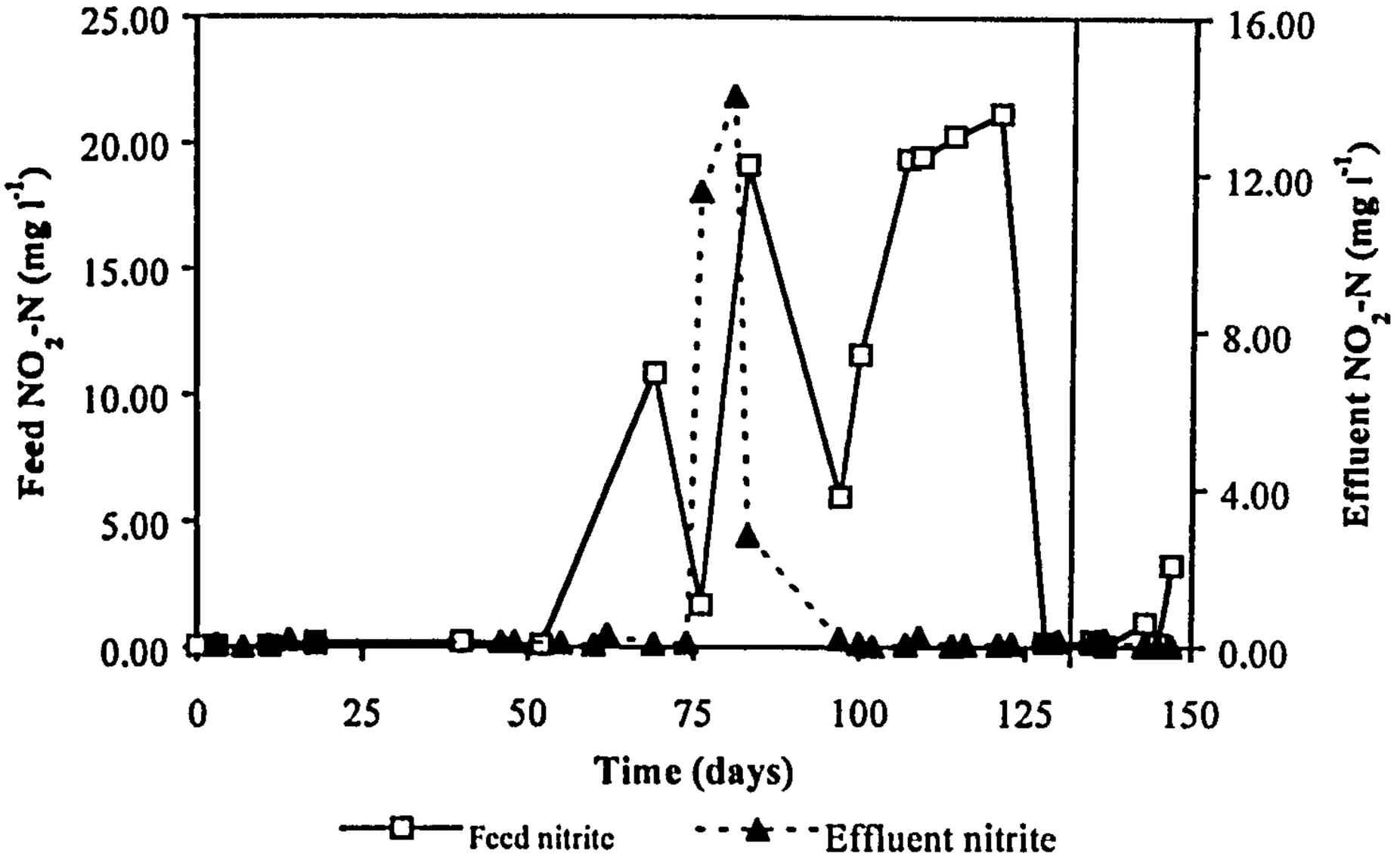


Figure 6.19. Feed and effluent NO_2-N concentrations for the duration of the study.

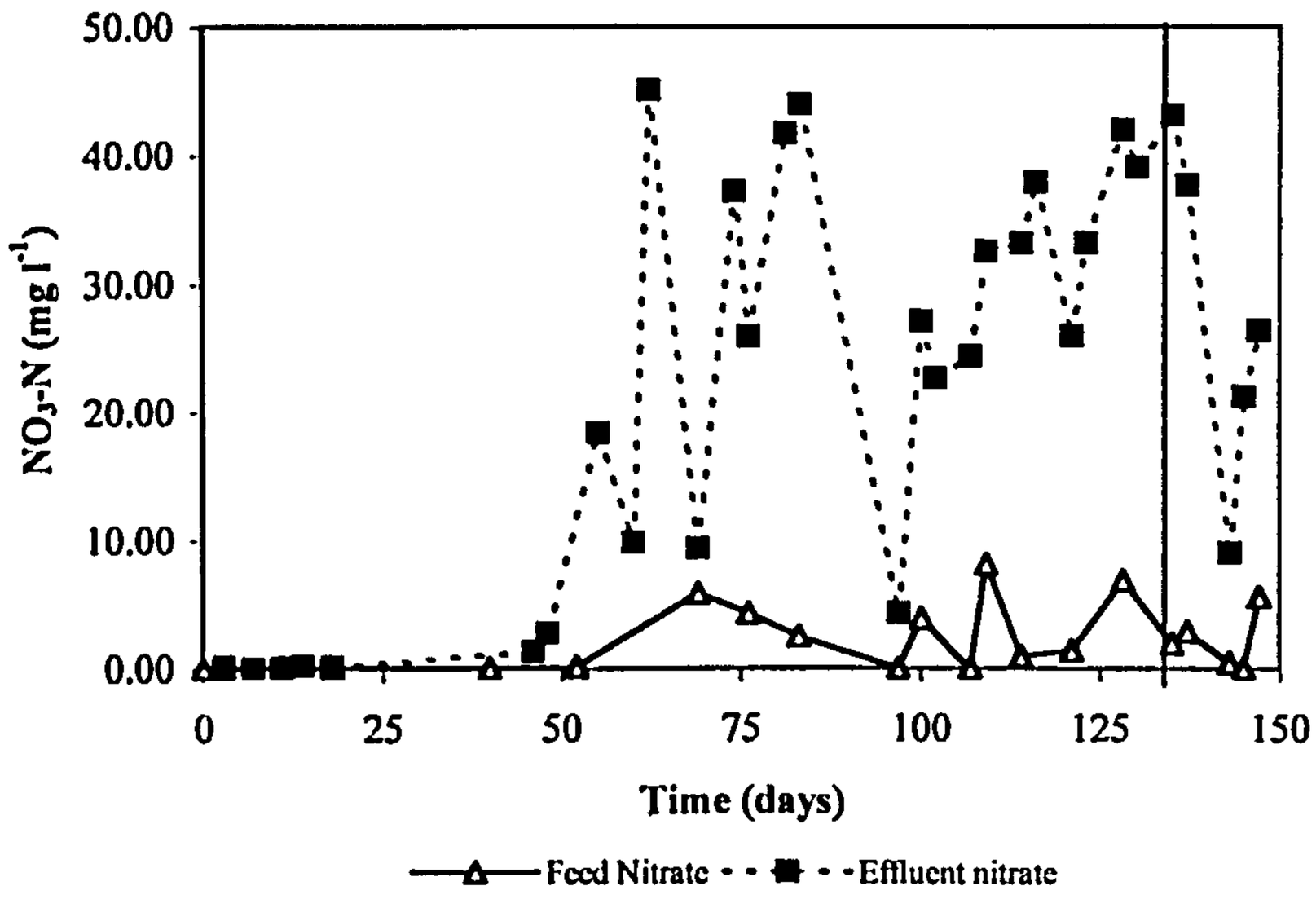


Figure 6.20. Feed and effluent NO_3-N concentration for the duration of the study.

6.4.3. Discussion

The nitrifying activity of the biofiltration unit was initially poor (days 0 - 48) until an effective biofilm formed. This was evident from an examination of the nitrification of NH_4-N with mean feed and effluent concentrations of 85.9 ± 10.95 and 29.6 ± 11.65 mg l^{-1} respectively. There was a noticeable decrease in effluent NH_4-N to 2.3 mg l^{-1} and concurrent increase in effluent NO_3-N from 0.68 to 29.16 mg l^{-1} after day 48, indicating the growth of nitrifying bacteria. There was a noticeable reduction in COD of *c.* 73% during this initial start up period (days 0 – 48). This may be expected because the biofilter is first colonised by heterotrophic bacteria responsible for carbonaceous oxidation, and subsequently by nitrifying bacteria. The growth of nitrifying bacteria is slow for an aerobic process due to the low yield of their energy producing pathways and hence the low rate of formation of new biological cells (Ødegaard, 1988; Hagopian and Riley, 1998). A further explanation would be the attachment of particulate material, with which the majority of the COD is associated, to the filter media. There was a concurrent reduction in SS of *c.*78%. This is also confirmed by the lower reduction in BOD between days 0 – 48 in comparison with days 48 - 134 with an increase from 60 to 91% in BOD reduction.

A N mass balance of the filter system reveals a reduction in N output in comparison with input. This may possibly be explained by removal of N by denitrification. The biofilm consists of both anoxic and aerobic zones. Anaerobic conditions are typically encountered in the deeper layers of the biofilm, where consumption of oxygen exceeds the rate of replenishment by diffusion. Under anaerobic conditions heterotrophic bacteria are capable of using NO_3^- as an electron donor instead of oxygen resulting in the conversion of NO_3^- to N_2 gas (Ødengaard, 1988; Metcalfe and Eddy, 1991). Other possible losses of N may

be due to volatilisation where ammonia is removed from the liquid to the gaseous phase as a result of high pH.

The increase in filter loading rate from 0.53 to 1.04 kg COD m^{-3} day^{-1} did not have an adverse impact on the nitrification process. Disregarding data during the start-up period (days 0 - 48), NH_4-N reduction was *c.* 98 and 99% with NO_3-N effluent concentrations of 27 ± 13 and 29 ± 12 mg l^{-1} for both loading rates respectively. The increase in loading rate did have a noticeable impact on the reduction of SS (Figure 6.15). The percentage removal of SS decreased from 87 to 79%. Similarly, there was a decrease in percentage BOD and TS removal of *c.* 10 and 3% respectively. The increase in organic loading did not however significantly alter the percentage reduction of COD. Considering that the majority of the COD load is associated with the particulate matter, it would be expected that an increase in effluent SS would result in a similar increase in effluent COD concentrations. Discounting the start up period, COD percentage reduction was *c.* 84 and 85% for both loading rates respectively. The reason for the similar reduction is not known.

The increase in loading rate also resulted in a reduction in reactive P removal by *c.* 20%. As demonstrated earlier, if the period associated with start up is discounted, this results in a reduction in percentage removal by a further 34%. The increase in organic loading resulted in approximately the same proportional decrease in P removal. The increase in effluent P concentration may therefore be due to the increase in reactive P release by heterotrophic bacteria with increasing load. P is not readily removed from conventional biological treatment processes. In order to perform this successfully, an additional treatment stage such as chemical precipitation or combined aerobic and anaerobic biological treatment systems would be required.

Chapter 7

Kinetic Study of the Anaerobic Digestion of Aquaculture Effluents at Psychrophilic and Thermophilic Temperatures

7.1. EXPERIMENTAL PROCEDURE

The ultimate CH_4 yield and rate of substrate utilisation were estimated as described in *Section 3.6*. Assay flasks were measured periodically for the production of biogas and excess biogas was wasted to prevent leakage due to excessive pressure. A glass syringe was connected to the gas vent on the assay flask and allowed to equilibrate to barometric pressure. The biogas was then analysed for composition as described in *Section 3.3.3*. The experiment was deemed finished when the production of CH_4 ceased (Once no production occurred after *c.* 15 days).

7.2. BIOCHEMICAL METHANE POTENTIAL TEST

Cumulative CH_4 yield for the aquaculture waste at a variety of VSS loadings, were tested together with the cellulose controls at 20 and 55°C (Figures 7.1 and 7.2).

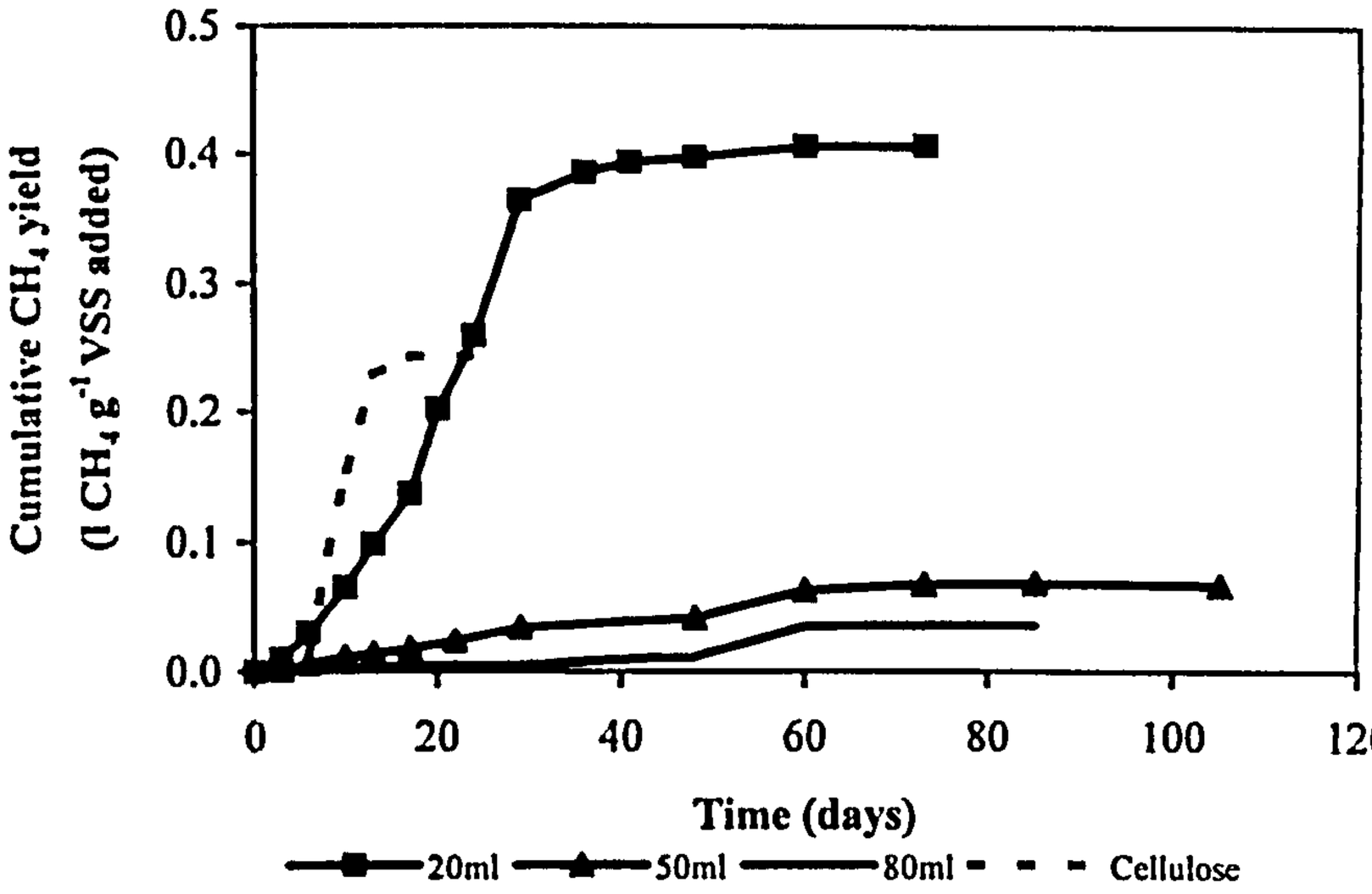


Figure 7.1. Cumulative CH_4 production of cellulose and various concentrations of aquaculture waste samples from the psychrophilic BMP assay.

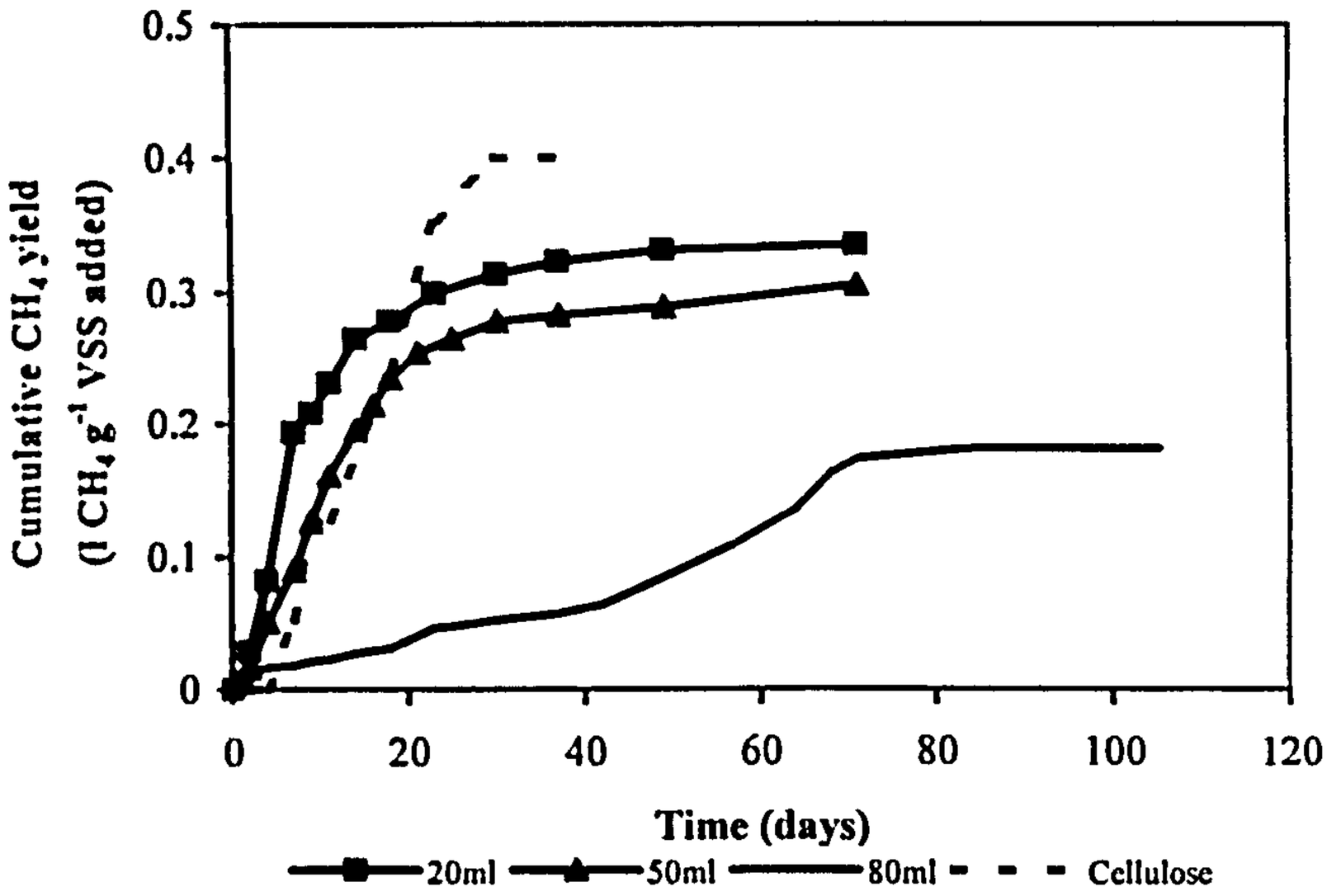


Figure 7.2. Cumulative CH_4 production of cellulose and various concentrations of aquaculture waste samples from the thermophilic BMP assay.

The ultimate CH_4 yield per gram of VS added for the cellulose controls under psychrophilic conditions was lower than the theoretical value (0.37 CH_4 m^3 kg^{-1} VS) and that from previous studies (Table 7.1). Similarly, the ultimate CH_4 yields for all samples were well below their predicted yields, with a decrease in the production of CH_4 with increasing VSS concentration. In contrast, the CH_{4max} at thermophilic temperatures was slightly higher than both the theoretical and literature CH_{4max} yield values.

Table 7.1. Range of biochemical CH_4 data from various feedstocks.

Feedstock	CH_{4max} (l g^{-1} VS added)	CH_4 rate constant (d^{-1})	Reference
Municipal solid waste	0.200 – 0.220	0.130 – 0.160	Chynoweth et al., 1993
Municipal solid waste	0.186 – 0.222		Owen and Chynoweth, 1992
Municipal solid waste	0.472		Cho et al., 1995
Water hyacinth	0.190 – 0.32	0.090 – 0.110	Chynoweth et al., 1993
Primary sludge	0.590		Chynoweth et al., 1993
Vegetable oil	0.940		Chynoweth et al., 1993
Bamboo	0.016		Chynoweth et al., 1993
Cellulose	0.370	0.140	Chynoweth et al., 1993
Cellulose	0.356		Cho et al., 1995
Cellulose	0.374	0.129	Owen and Chynoweth, 1992

The BMP assay adheres to first order kinetics. Deviation from this profile can, however, occur due to the presence of fast or slow digestible components (e.g. soluble and structural carbohydrates) and inhibitors (Chynoweth et al., 1993). This is evident from an examination of the data from both the psychrophilic and thermophilic assays. At both assay temperatures, there is a general decline in the both the CH_4 yield and the rate of CH_4 production as a result of an increase in the concentration of VSS added. In *Section 2.7*, the shape of the curves for feed volumes of 20 ml for the psychrophilic, and 20 and 40 ml for the thermophilic are predicted by the first order equation:

$$CH_4 = CH_{4\max}\left[1-\exp^{-kt}\right] \quad \text{..... (Equation 7.1)}$$

The slopes of these curves decrease with time, indicating a gradual decrease in the concentration of biodegradable substrate. It is clear, however, from Figures 7.1 and 7.2 that for feed volumes in excess of 20 ml for the psychrophilic assay and 50 ml for the thermophilic assay the curves no longer fit the exponential model. Therefore, for feed concentrations in excess of 3.4 and 5.6 kg m^{-3} for the psychrophilic and thermophilic assays, the experimental data do not fit the first order kinetic model.

For experimental data which conformed to the first order model (Figure 7.3), it was possible to evaluate the kinetic constants corresponding to the rate of CH_4 production and thus the rate of biodegradable substrate utilisation. Correlation coefficients (R^2 values) indicate a high degree of applicability of the experimental data to the first order model. The R^2 value was greater than 0.9 for all three data sets.

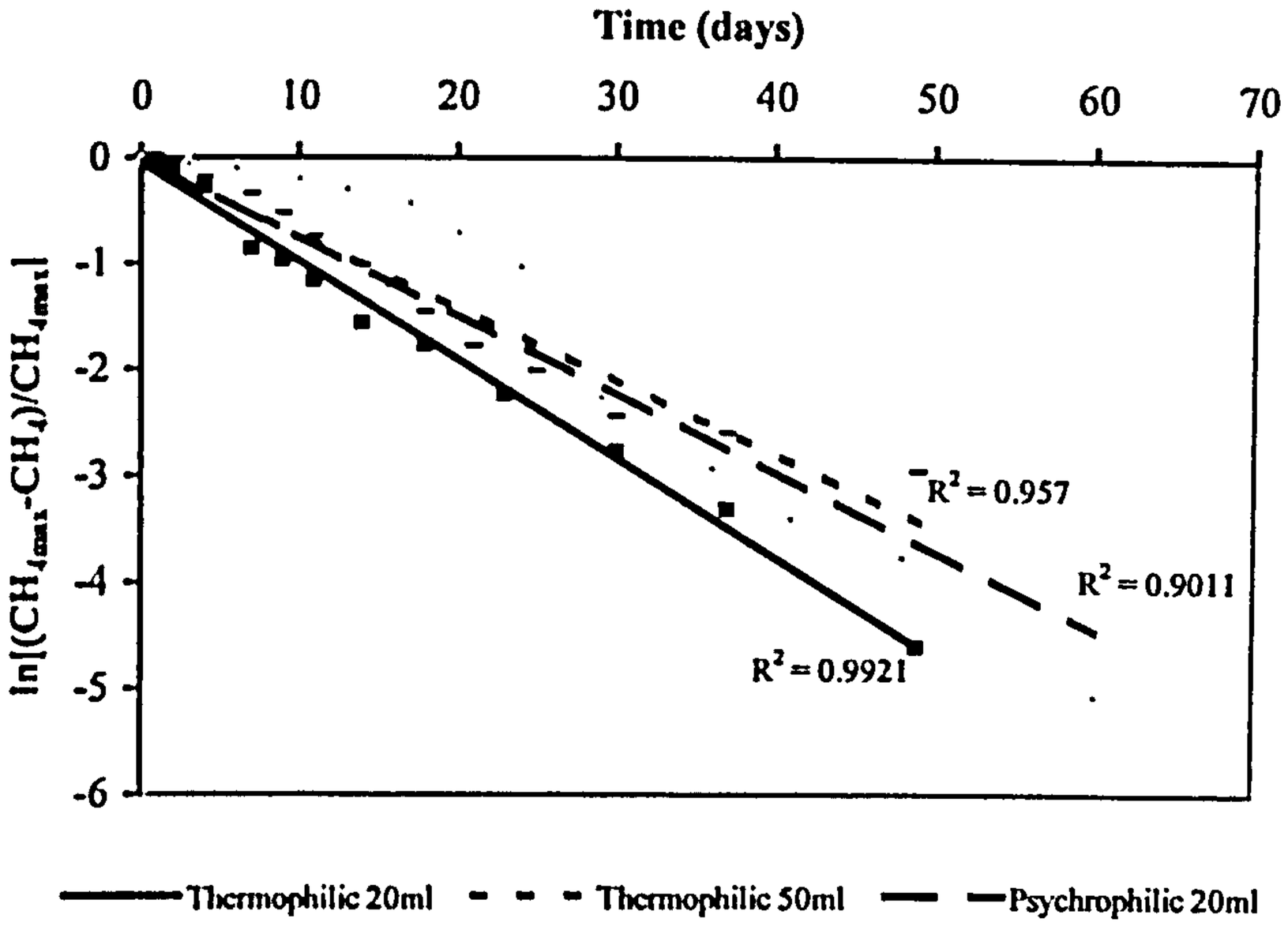


Figure 7.3. Variation in the Ln[(CH_{4max}–CH_4)/CH_{4max}] for varying feed concentrations with temperature and time.

7.3. DISCUSSION

The ultimate CH_4 production for the cellulose controls varied at both operating temperatures and was significantly lower at psychrophilic temperatures than the predicted theoretical yield (Table 7.2). The CH_{4max} g^{-1} VSS added was close to the predicted theoretical yield at thermophilic temperatures, but it was not possible to determine why this value was much lower at psychrophilic temperatures.

Table 7.2. Biochemical methane potential data and theoretical CH_4 yield in this study.

Assay feed concentration (g VSS l^{-1})	Assay Temperature (°C)	CH_{4max} (l g^{-1} VSS added)	CH_4 rate constant (d^{-1})	Theoretical CH_{4max} yield (l g^{-1} VSS added)
3.35	20	0.410	0.1030	0.59
8.45	20	0.067	Biphasic	0.59
13.50	20	0.036	Biphasic	0.59
20.30	20	---	—	0.59
Cellulose 0.6	20	0.243	Biphasic	0.37
2.23	55	0.334	0.0930	0.59
5.63	55	0.305	0.0695	0.59
9.00	55	0.182	Biphasic	0.59
13.53	55	---	—	0.59
Cellulose 0.4	55	0.401	Biphasic	0.37

It is evident that an increase in feed VSS concentration above 3.4 and 5.6 g l^{-1} at psychrophilic and thermophilic temperatures respectively results in a decrease in CH_4 production and also the rate of CH_4 yield (Figures 7.1 and 7.2). The profile of these curves demonstrates the biphasic conversion of biodegradable substrate to CH_4, indicating inhibition of the biochemical process (Chynoweth et al., 1993). Sanchez et al. (1996) also demonstrated the reduction in CH_4 production rate with the increase in loadings of sugar mill mud waste to a completely mixed digester. The digestion process conformed to first-order kinetics, although at substrate concentrations greater than 29.5 g COD l^{-1}, the data did not fit the first order kinetic model. The decrease in the CH_4 production rate was attributed to substrate inhibition.

Chynoweth et al. (1993) investigated the factors influencing the validity of the BMP assay. They demonstrated the importance of the inoculum to feed ratio (I/F) showing an increase in the rate of CH_4 produced with an increase in the I/F ratio. It was therefore suggested that for the analysis of some biomass samples in the BMP assay, the I/F ratio

may need to be increased from approximately 1 to 2 (VS basis). The I/F ratio for the psychrophilic and thermophilic assays was 0.5 and 0.3 respectively, much lower than standard literature values. With an increase in VS substrate concentration, accumulation of VFA may occur due to the faster growth rate of hydrolytic bacteria in comparison with methanogenic bacteria. As the VFA concentration increases, the pH of the digester liquor will decrease, possibly to the point at which methanogenic bacteria will die off.

The particle size of the substrate has been shown to influence the validity of the BMP test (Chynoweth et al., 1993). As particle size is reduced, hydrolysis of the substrate and solubilisation of organic material would be expected to increase (*Section 2.10.2*). Samples for analysis are generally passed through an Urschell mill equipped with a 0.8 mm head. However, no pre-treatment of the aquaculture waste to alter its particle size occurred in this study. This may have resulted in a slower rate of degradation and hence CH_4 production due to the adhesive nature of aquaculture waste and thus the larger particle sizes present in the assay. This should not affect the overall CH_{4max} yield, and the failure of any assay in terms of CH_4 production must be attributed to the occurrence of inhibition.

Chapter 8

The Economic Viability of Anaerobic Digestion for Aquaculture Systems

8.1. INTRODUCTION

In *Section 2.12.1*, the need for an increasing demand for the production of energy from renewable sources was discussed. Anaerobic digestion of organic material results in the production of a biogas consisting mainly of CH_4 which can be burned to allow energy recovery either through the generation of electricity or production of heat. Furthermore, residual solid material from the digestion process may be utilised as a soil conditioner and digester liquor as a fertiliser.

The economic viability of an anaerobic digestion system will depend largely on the ability or motivation to utilise its full potential in terms of its beneficial products. The capital and operational costs involved will vary depending on the degree of beneficial product utilisation. The nature of the feedstock will also influence the capital costs of an anaerobic degradation system and its potential product usage. However, agricultural wastes are highly suitable for anaerobic digestion and offer the potential to form saleable compost.

The quantity of waste available for digestion will also be a large factor in determining the financial success of the digestion system. The greater amount of degradable waste available will result in a greater production of biogas and material which may be utilised as beneficial by products. Availability of waste material for the digestion process will

depend largely on the ability to recover the waste from the production process. For example, waste slurry from cattle may be readily recovered from animal housing during winter feeding but the quantity available during much of the remaining year will be reduced when animals are put to pasture. Similarly, land based aquaculture units, due to their contained nature, will result in a greater ability to recover waste than cage aquaculture systems. Ultimately the quantity of waste available for digestion will depend on the technology available for waste recovery.

The objective of this analysis was to examine the economic viability of an anaerobic digestion unit for the treatment of waste aquaculture effluents. A number of potential scenarios were studied in order to evaluate the most economically viable option for the development of anaerobic digestion as a waste management technology for aquaculture.

8.2. METHODOLOGY

8.2.1. Calculation of Waste Output

Care is needed in the calculation of wastage rates from the farm, as they will directly influence the design and utilisation of the anaerobic digestion system. Estimates of solid waste outputs from the Loch Earn fish farm site were based on daily feeding rates and calculated using a feeding – waste model (Cromey, 2000). Feed input will vary greatly depending on factors such as water temperature, stocking density and farm production rate. Therefore, average feed input values were taken from previous years and it was estimated that there was a daily feed input range of 1,025 – 1,500 kg. The lower feed input (FI) estimate was considered in order to obtain a "worst case" scenario for the economic viability analysis. Specifications for digestibility (Fd_{dig}) were obtained from the feed manufacturer, while moisture content (Fd_{wat}) of the feed was estimated from

measurements obtained throughout the monitoring of the waste collection system at Loch Earn. Feed digestibility and moisture were estimated to be 89 and 6.7% respectively. Feed wastage (Fd_{was}) was considered to be between 0% and 5%. A middle range value of 3% was therefore taken. Faecal production rates (F_{ae}) were calculated as follows:

$$F_{ae} = (1 - Fd_{wat}) \times (1 - Fd_{dig}) \times (1 - Fd_{was}) \times FI \quad \text{.....(Equation 8.1)}$$

Where food wastage rates (F_d) are represented in the model by:

$$F_d = Fd_{was} \times (1 - Fd_{wat}) \times FI \quad \text{.....(Equation 8.2)}$$

With a daily food input value of 1,025 kg, the food wastage rate was calculated as 28.5 kg and the faecal wastage rate was calculated as 102 kg. Therefore, the total waste output from the farm was estimated as *c.* 130 kg day^{-1}. However, the quantity of waste available for anaerobic digestion would depend on the operational efficiency of the waste collection system. In order to investigate the potential of anaerobic digestion, the economic viability of the system was considered to have a waste collection efficiency of 100%.

8.2.2. Anaerobic Digestion System

The basic outline and options for an anaerobic digestion system for an aquaculture farm are shown below (Figure 8.1). The main components of the system are the anaerobic digester itself and a biogas utilisation device such as a boiler or generator. A dewatering unit is required due to the high water content of the waste. A suitable waste concentration must first be achieved for optimal loading of the digester. Once the digestion process has been completed, waste effluent may be discharged via a biofiltration device. Undecomposed solid material or digestate may be aerobically composted and sold as a general-purpose compost. This will require a separator and composting equipment.

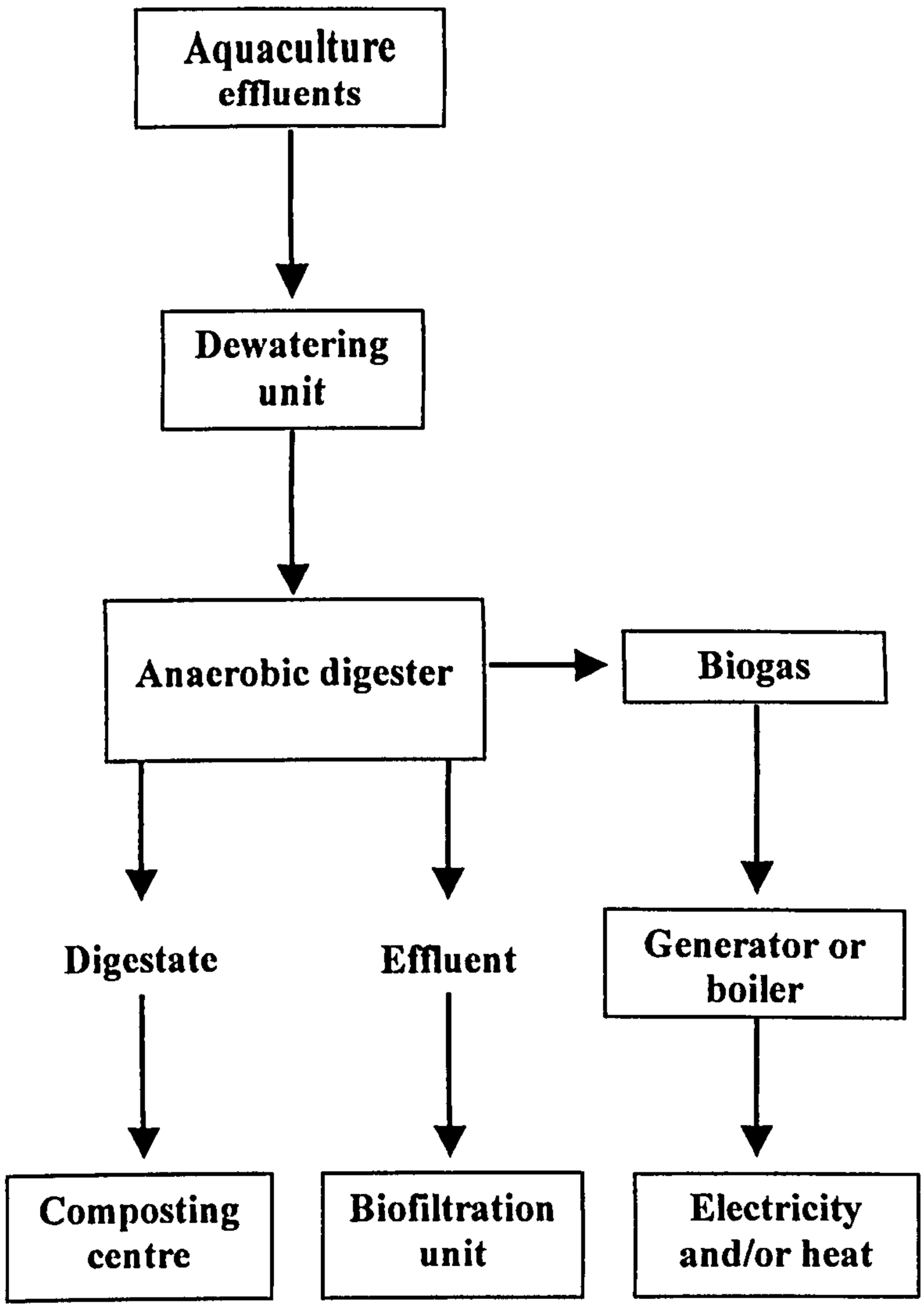


Figure 8.1. Schematic of the anaerobic digestion system for aquaculture waste effluents.

8.2.3. Capital Costs

Depending on the degree of utilisation of digestion products, the capital costs will vary. In addition, the type of digestion system to be used will change. A conventional solids anaerobic digestion system and a specialised patented digester designed towards providing material directly for compost (SWAP system™) were considered.

8.2.3.1. Anaerobic digester

The cost of the anaerobic digester will depend primarily on the required size. The size was calculated from an estimation of the waste output from the farm and from experimental data obtained in *Chapter 5* and *Chapter 6*. Typical loading rates for anaerobic digesters range between 0.8 – 3.2 kg VS m^{-3} day^{-1} (Kiely, 1997). Based on these typical loading rates, a middle range loading rate of 2 kg VS m^{-3} day^{-1} was chosen for this system. Although it would lead to a larger digester system, it is better to oversize than to undersize (Boyd, 2000). Laboratory analysis revealed that aquaculture effluents consisted of *c.* 80% VS. The required anaerobic digester size may therefore be calculated as follows:

130 kg waste day^{-1} × 80% VS × 100% collection efficiency = 104 kg VS day^{-1}

At an organic loading rate of 2 kg VS m^{-3} day^{-1}, a digester of approximately 55 – 70 m^3 would be required, allowing for headspace gas (104 kg VS day^{-1} / 2 kg VS m^{-3} day^{-1}). A number of digester manufacturers were contacted and quotes were obtained. Prices ranged from £50,000 to £70,000. For this analysis the cheapest quotes were used. Two types of digestion system were considered, based on the use of the end products. A conventional solids anaerobic digestion system was estimated at £50,000 from Livestock Systems Ltd. This was inclusive of heating (mesophilic) and mixing units and also the boiler system. Effluent from the digester could then be further treated or "polished" using a simple aerobic biofilter.

The second digester system considered was a SWAP system™. This unit is specifically designed for the production of compost. Aquaculture waste is premixed with a fibrous material such as hay or straw before loading to the digester. On completion of the

digestion process, the digestate is directly suitable for composting. The fully installed and commissioned price for the patented SWAP system™ from Safe-Waste Systems UK Ltd., Newcastle Upon Tyne, would be £48,000 inclusive of pre-mixing equipment, generator, boiler and pumps.

8.2.3.2. Composting equipment

The capital costs involved for the production of compost were taken from literature sources based on existing successful ventures mainly associated with digestion of pig slurry (Boyd, 2000). Using a conventional digestion system would require separation of the digestate fibre and liquor. Literature values of separator cost are *c.* £20,000. In addition, a turning device would be required for the production of compost and this was estimated at £16,500. In light of these costs, it was decided that the production of compost using a conventional digestion system was economically unfeasible. The SWAP system™ was therefore the only system considered in the analysis where the composting of digestate was to be exploited as a means of revenue.

Additional equipment required for the production of compost includes polytunnel, estimated cost £500, and a bagging machine. Although not essential, the bagging machine was considered to enhance the profitability of the compost, based on existing enterprises with pig farm slurry. The estimated cost of a bagging machine is £4,000, although a second hand device could be purchased for *c.* £1,500. For this analysis, the cost of a new machine was considered.

8.2.3.3. Combined heat and power (CHP) unit

A CHP unit is designed for the production of heat and electricity from biogas. However, the CHP unit is specialised equipment and literature prices range from £25,000 to £75,000. Alternatively, a cheaper means of generating power from biogas is the use of a diesel generator, the cost of which is *c.* £4,000. The diesel generator is a less efficient means of generating electricity in comparison with the CHP unit, probably less than half or below 20%. However, based on capital cost alone, the CHP unit would only be suitable for large scale systems such a community incinerator. The diesel generator also has the added advantage of not being corroded by the biogas due to the use of diesel, which acts as a lubricant. Furthermore, the generation of heat within this case study is not considered as beneficial as electricity. Thus, the use of a CHP unit was not considered relevant to the study.

8.2.4. Operational Costs

Similar to the use of digestion systems on pig farms, it is also considered here that daily maintenance of the digestion system could be incorporated into the existing routine of the aquaculture farm. Operational and maintenance costs were taken from literature, based on existing similar enterprises. Using these estimates, the operational costs for a 70 m^3 digester were compiled (Table 8.1; scenarios defined in Table 8.2). Operational costs defined include the cost of digester heating, feeding and maintenance.

Table 8.1. Capital and operational costs for the differing scenarios applied to the economic viability analysis.

	Scenarios 1 & 2 (£)	Scenarios 3 & 7 (£)	Scenarios 4 & 8 (£)
Capital Costs			
Anaerobic system	50,000	45,000	48,000
Patent license	N A	1,700	3,400
Dewatering system (Drum Filter)	3,385	3,385	3,385
Storage Tank	10,000	N A	N A
Diesel generator	4,000	N A	N A
Biofilter	3,500	N A	N A
Polytunnel	N A	500	500
Bagger	N A	4,000	4,000
Total Capital costs	70,585	54,585	59,285
Expected Annual Revenue			
Electricity	1,400	1,400	1,400
Compost		2,000	4,000
Gross Operational Costs	1,000	2,000	3,000
Operating Profit	400	1,400	2,400

Note: Installation costs are included in the cost of the digestion system.

8.2.4.1. Calculation of income stream

The profits associated with use of anaerobic digestion may be categorised into two main areas, energy production and the sale of compost.

8.2.4.1.1. Electricity production

Electricity generation will be affected primarily by the quantity and composition of biogas produced. Similarly, biogas production will be affected by the rate of VS destruction, if it assumed that VS degradation is directly related to the production of

biogas. Therefore, an estimation of the quantity of biogas produced was made from laboratory studies and the following mass balance calculation (Boyd, 2000):

Density of $CH_4 = 0.71$ kg m^{-3}

Density of $CO_2 = 1.96$ kg m^{-3}

Biogas composition of 60% CH_4 and 40% CO_2:

$$1\ m^3 \text{ biogas} = (60\% \times 0.71\ \text{kg}\ m^{-3}) + (40\% \times 1.96\ \text{kg}\ m^{-3})$$

$$= 1.21\ \text{kg}$$

At VS reduction efficiencies of 60% and assuming 1 m^3 biogas equals 1.21 kg VS:

$$104\ \text{kg VS day}^{-1} / 1.21\ \text{kg} \times 60\% = 52\ m^3 \text{ biogas day}^{-1}.$$

Pure CH_4 has a heating value of 33,810 kJ m^{-3}. It was therefore assumed that biogas with a composition of 60% CH_4 would have a heating value of 20,286 kJ m^{-3}. The total energy value of the biogas from the anaerobic digestion system would therefore be *c.* 1.05 million kJ day^{-1}.

It was further assumed that a typical internal combustion engine used to generate electricity requires 13,650 kJ to generate one kilowatt hour (kWh) of electricity (*c.* 26% efficiency). This implies that the annual kWh generated would be *c.* 28,207 kWh, assuming the generator was in continuous operation. If the electricity produced were sold at commercial rates (5 pence per kWh) it would have a value of *c.* £1,400.

8.2.4.1.2. Compost sales

The annual benefits from the sale of compost material were based on comparisons from existing ventures involving the use of anaerobic digestion on pig farms. Using the SWAP system™, it is estimated that 2 t of material, for composting per week could be produced from a feedstock containing 50% fibrous material such as hay or straw (Whaley, pers comm). Boyd (2000) cited the use of a bagging machine by a pig farmer in the UK enabling him to sell 15 kg bags of compost at £4.80 per bag. If a conservative value of £1 per 15 kg bag of compost was used, it is estimated that the SWAP system™ could produce the equivalent of *c.* £6,900 worth of compost annually. However, this is highly dependent on the marketing of the product and public perception. Another estimate obtained for the value of the compost, although unbagged, was only £10 t^{-1} (Boyd, 2000; Whaley, pers comm.). This is the equivalent of only £1,040 annually. Taking into consideration the cost of marketing, transport and retail costs, and also the reduction in weight and volume of the waste material during composting, a conservative value of £2,000 per annum was used in the analysis in conjunction with the purchase of a bagging machine and a viable market.

8.2.5. Other Factors

Although not considered in this analysis, other factors such as gate fees may increase the rate of return on the investment. With the increase in the Landfill Tax (presently £ 12 t^{-1} for non-inert/active waste and increasing by £1 t^{-1} $year^{-1}$ until 2004; HMCE, 2000), organic waste will have a negative value for the business sector (Boyd, 2000). In return for a gate fee lower than the landfill tax, the digestion system may be a useful alternative for the disposal of organic wastes. It has been estimated that an annual income of up to £6,000 may be achieved (Higham, 1998). However this will greatly depend on the

surrounding community and business sector. It would also preferably require the purchase of pasteurisation equipment, especially for the treatment of organic waste from the food industry, which would increase capital costs by a further £12,500 (Boyd, 2000).

Furthermore, there would also be a decrease in waste disposal costs for the farm. Although not a consideration at present for cage farms, if a waste collection system were installed then it is estimated that at 100% collection efficiency and assuming 100% dewatering, the annual cost of slurry disposal to landfill would be an estimated £14 - 15,000 (BIFFA Waste Services Ltd.) based on disposal rates of £35 t^{-1} with labour rate of £65 $hour^{-1}$). This greatly exceeds the cost of an anaerobic digestion system over the 15 year period considered here.

8.3. ECONOMIC ANALYSIS

In order to examine the economic viability of the digester system and its optional components, a cost benefit analysis was undertaken. Initial capital costs and investments were determined together with operational costs and resulting profits for the lifetime of the project. The typical lifetime of an anaerobic digestion unit is 15 – 20 years. A conservative estimate of 15 years was used in this analysis. The economic viability of an investment for any business will greatly depend on whether the net present value (NPV) of the project is positive. The return on investment will occur when the NPV is zero. Discount rates are applied to represent the depreciation of money through time. Discount rates are often based on the nominal risk free yield on government bonds, which are typically between 5.4% and 7.3% per annum across the EU. Meeks and Bates (1999) used this rationale and applied a discount rate of 6%. They also took into consideration the possibility that an investment in such abatement technology would displace other higher

risk investments with a higher rate of return, thus a higher discount rate of 15% was considered. Similarly, discount rates of 6% and 15% were applied in this analysis. The discount rate for which the NPV is zero is termed the internal rate of return (IRR). It is the maximum interest rate at which an investment should be made (i.e. an investment should not be made if the IRR is lower than the interest rate or discount rate) (Craven, 1984). The NPV was calculated as follows:

$$Net\ present\ value = \frac{Future\ sum}{(1+d)^n} \quad \text{.....(Equation 8.3)}$$

Where:

d = discount rate;

n = number of years of discounting.

8.3.1. Scenarios

In carrying out a cost benefit analysis of an anaerobic digestion system, a variety of scenarios were considered in order to determine the most, if any, economically viable option (Table 8.2). Firstly, a conventional anaerobic unit with biofiltration for disposal and generator for electricity production was considered. Secondly, the same system was analysed with the aid of government funding. Many similar systems in the UK have received a 50% grant due to their contribution towards renewable energy. Also, a third scenario with a different digestion system (SWAP system™) was analysed with a view to increasing profits as a result of compost sales. The same system was considered with an increase in waste input with the view to increasing compost productivity. Furthermore, as with scenario 2, both the latter systems were analysed with the aid of government funding. All 6 scenarios used a discount rate of 6% and if profitable, were examined

using a discount rate of 15%. Finally, scenarios 11 - 14 examine the feasibility of the project using optimum sales values for compost.

Table 8.2. Summary of varying scenarios for the analysis of anaerobic digestion as an economically viable option for the treatment of aquacultural effluents.

Scenarios	Description
1	Conventional AD system electricity production and biofiltration system
2	Scenario 1 with 50% grant
3	SWAP system™ with electricity and compost production at 2 t loading
4	Scenario 3 with 4 t loading
5	Scenario 3 with 50% grant
6	Scenario 4 with 50% grant
7	Scenario 3 at discount rate of 15%
8	Scenario 4 at discount rate of 15%
9	Scenario 5 at discount rate of 15%
10	Scenario 6 at discount rate of 15%
11	Optimum values for compost sale at 2 t loading
12	Scenario 11 at 4 t loading
13	Scenario 11 with 50% grant
14	Scenario 12 with 50% grant
15	Scenario 13 at 15% discount rate
16	Scenario 14 at 15% discount rate

8.4. RESULTS AND DISCUSSION

The economic viability analysis clearly showed that certain parameters had a strong influence on the viability of the project (Table 8.3). It is also evident that in order for the system to be financially viable, exploitation of its beneficial products must be maximised. This is demonstrated with the choice of digestion system to be used. A conventional

anaerobic digestion unit will not be suitable for the production of compost due to the large capital investment required. The only means of selling revenue is therefore through the production of electricity. The annual income generated as a result of electricity production alone would be *c.* £1,400. A greater mass of waste and hence larger digester would be required in order to generate sufficient electricity to allow the system to become financially viable. It is clear that scenarios 1 and 2 (electricity production) are not viable as a means of generatory income revenue (Figure 8.2). IRR was calculated at –21.71% and even with the aid of a 50% grant (Figure 8.2, scenario 2), the IRR remains negative at –16.85%.

Table 8.3. Summary of results calculated from the economic viability analysis.

Scenario	NPV (6%)	NPV (15%)	IRR	Payback Year (6%)	Payback Year (15%)
	£	£	%		
1	-67,000	NA	-21.71	N A	N A
2	-31,558	NA	-16.85	N A	N A
3 & 7	-40,987	-46,398	-10.10	N A	N A
4 & 8	-35,975	-45,251	-5.67	N A	N A
5 & 9	-13,194	-19,105	-3.11	N A	N A
6 & 10	-6,332	-15,608	2.53	N A	N A
11	-11,851	NA	2.47	N A	N A
12	7,729	NA	7.94	13	N A
13 &15	15,441	-3,063	13.80	8	N A
14 & 16	37,372	10,704	21.11	6	8

Note: N A = not applicable.

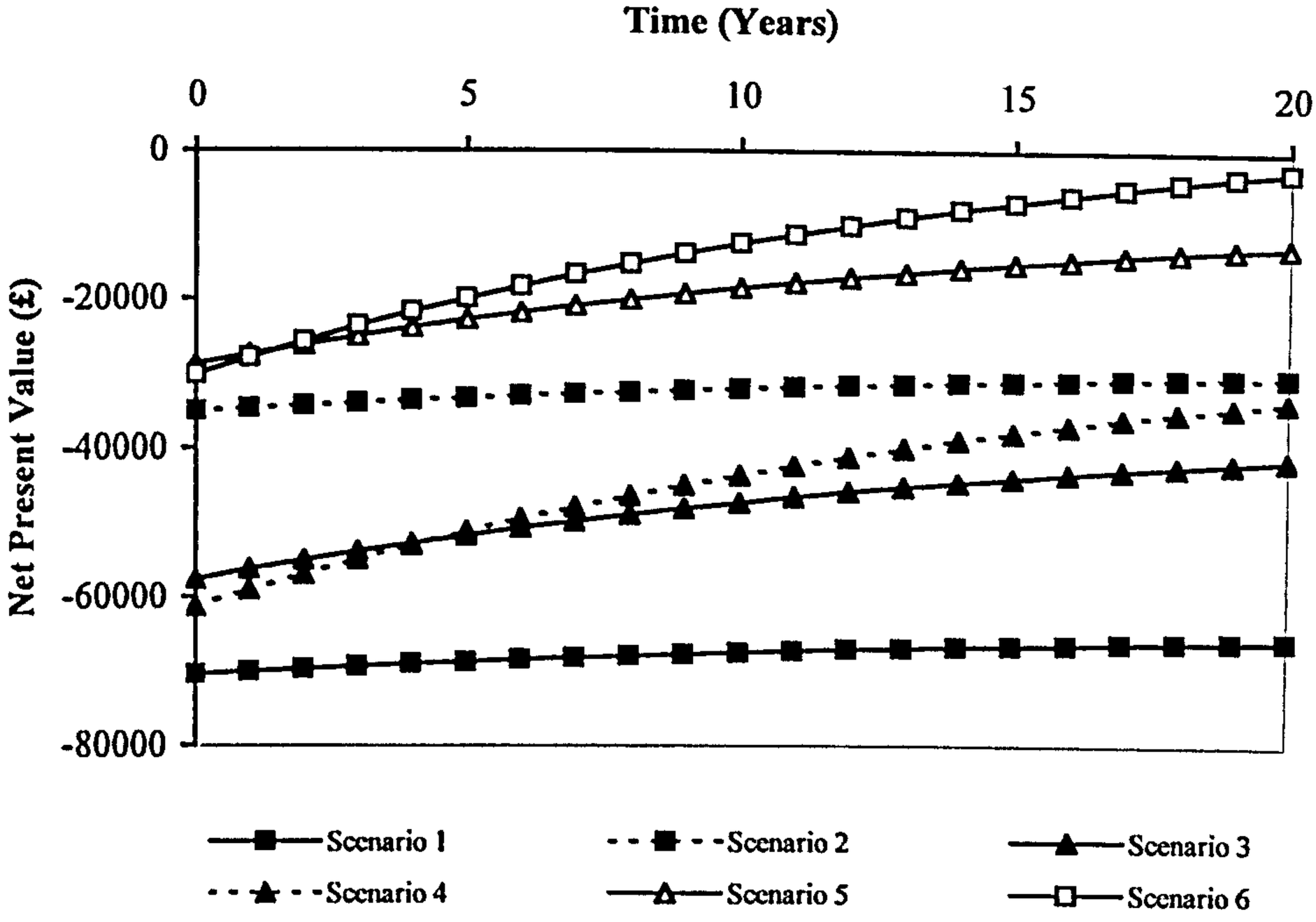


Figure 8.2. Economic viability of anaerobic digestion for aquaculture at a discount rate of 6%.

The SWAP system™, which is designed to offer greater potential for composting, had a similar unattractive IRR at -10.10%, with a NPV of -£40,987 after 15 years (Table 8.3). Although electricity production is not improved, scenario 3 shows that the ability to sell the resulting compost can increase revenue by *c.* £1,000. Furthermore, the profits of this system could be improved by doubling the load to the digester with only a slight increase of *c.* £5,000 and £1,000 in capital and operational costs respectively. However, the resulting NPV after 15 years at a discount rate of 6% remained negative at -£35,975. Even with the aid of a 50% grant, the NPV for the SWAP system™ with the higher loading of 4 t week^{-1} remains negative at -£6,332 (Figure 8.2). Consequently, using the higher discount rate of 15% in the analysis, the project, as expected, becomes even more financially unattractive (Figure 8.3, scenarios 7 – 10).

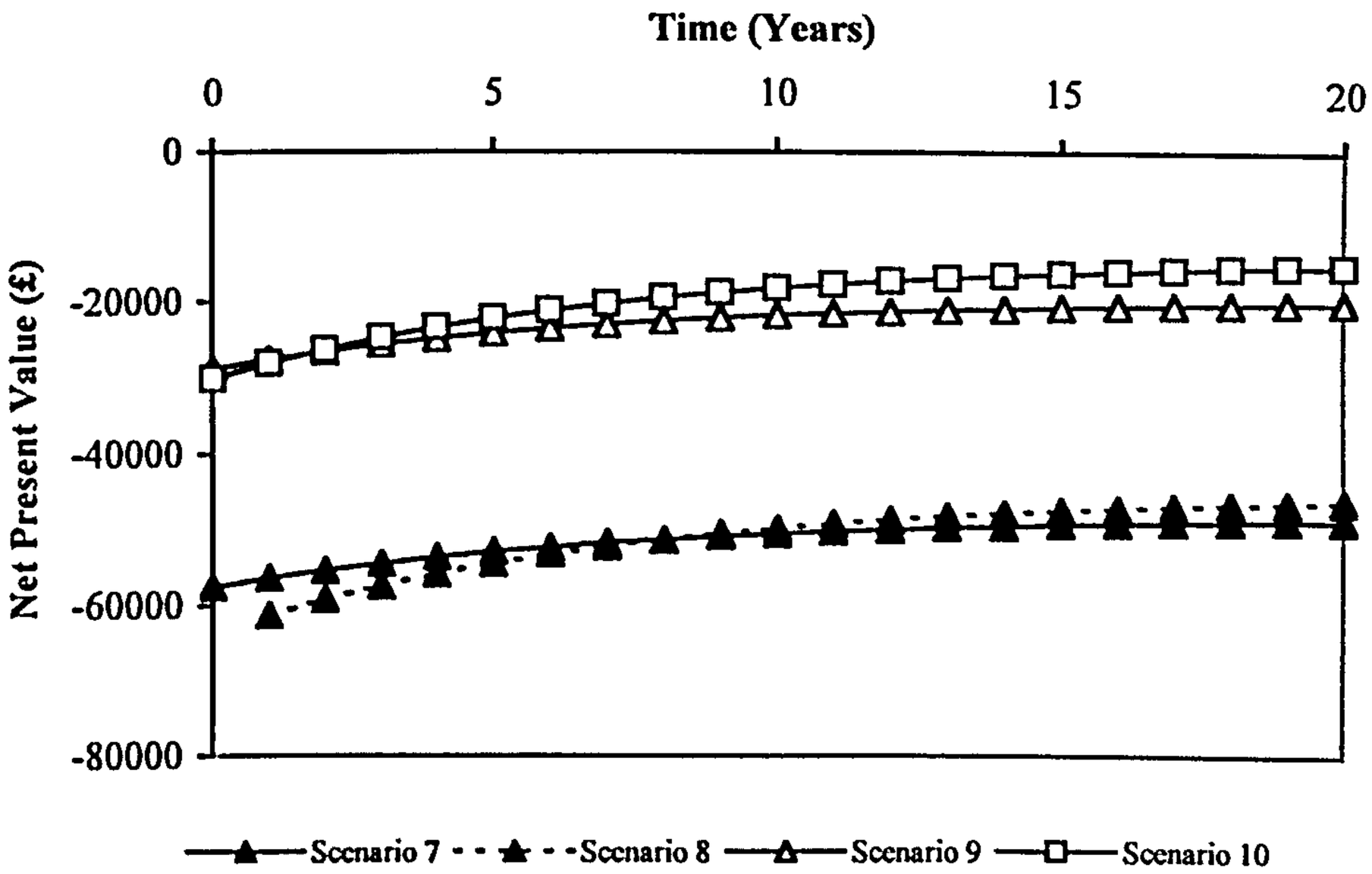


Figure 8.3. Economic viability of anaerobic digestion for an aquaculture farm at a discount rate of 15%.

It can be seen that the economic viability analysis is extremely sensitive to the ability to obtain grant money and fully utilise the products of the digestion process. Although, the NPV remains negative for scenarios 1 – 10, the IRR for all scenarios rises with the increase in financing from a 50% grant and extra revenue generated from the sale of compost. The IRR rises from –21.71% (scenario 1) to –10.1% (scenario 3) with the sale of compost and a further rise to –3.11% (scenario 5) when a grant is taken into account.

When compared to the costs of transporting the untreated aquaculture waste to landfill, the anaerobic digestion system appears to be a more favourable option. If it is assumed that the concentration of the untreated waste effluent from the collection system operating at 100% efficiency is similar to that of cattle slurry at *c.* 0.05 kg l^{-1} (estimated from laboratory analysis, *Section 6.3*, and Kiely, 1997), then the total weekly waste load would be *c.* 18,200 l. It may also be assumed that there would be 3 h labour costs associated

with collection and transport off-site. Using a 20,000 l tanker, the annual cost can be estimated at *c.* £50,000. When compared to the costs of the anaerobic digestion system, this is extremely high. However, it may be safely assumed that all of the waste would not be transported to land fill with land spreading instead being a more practical option.

The sensitivity of the analysis towards product utilisation and grants can be further demonstrated in scenarios 11 – 16 (Figure 8.4). If the value of the compost is increased to its optimum sales value of £6,900 per annum and using £1,900 for marketing the product, the expected revenue after operational costs would be £4,400. The resulting NPV, although still negative at -£11,851 (scenario 11) is a vast improvement when compared to the same analysis using a lower compost sale value (scenario 3). Similarly, an increase in the waste load to the system (scenario 12) makes the project more economically attractive allowing payback within 13 years and an NPV of £7,729.

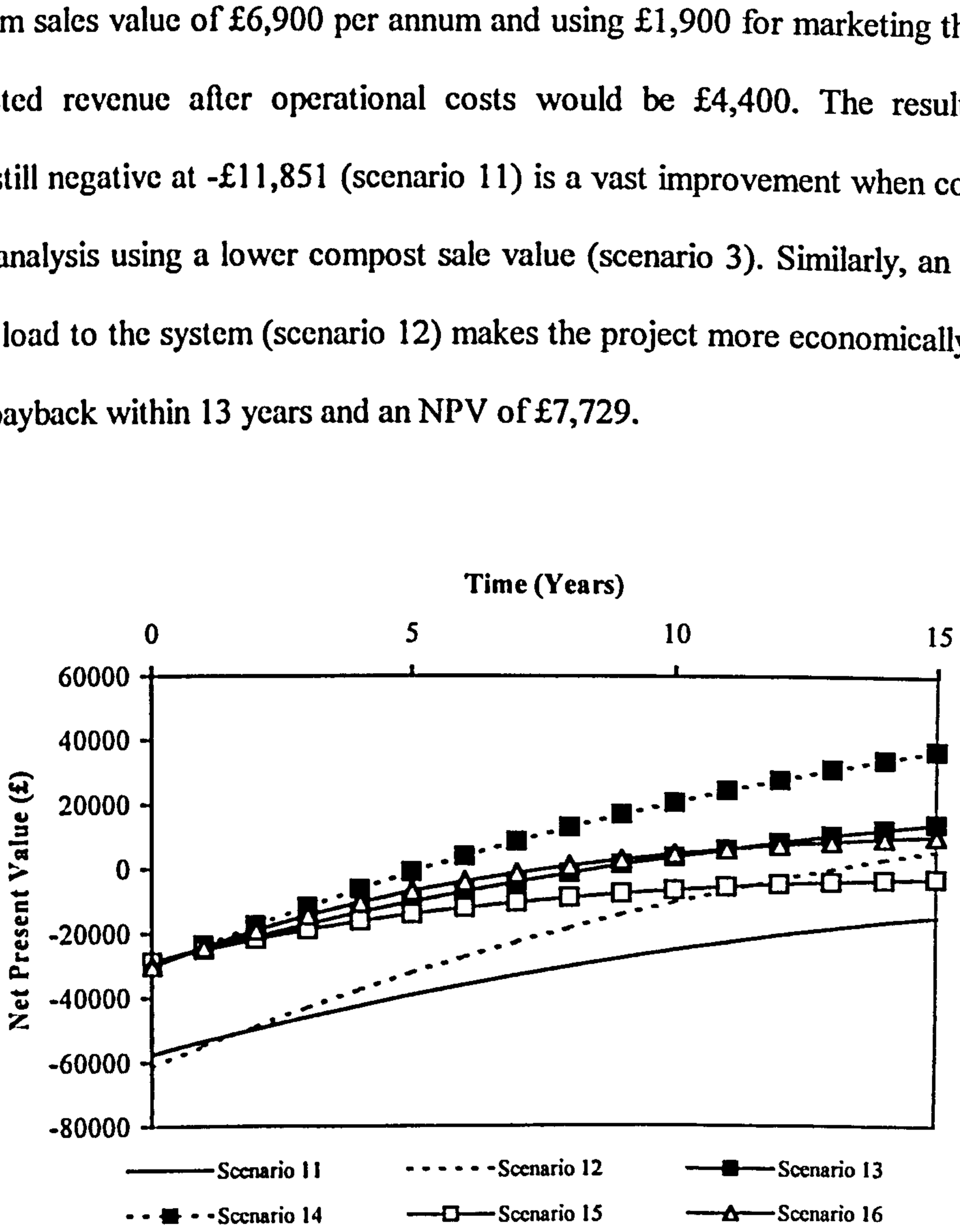


Figure 8.4. Economic viability of anaerobic digestion for aquaculture at optimum conditions.

The use of anaerobic digestion as a means of waste management for a single farm unit similar in production size to that of the study site at Drummond fish farm, appears to be financially unattractive. The products of the digestion processes would have to be utilised to their full potential with the economic success of the project dependent strongly on the market price obtained for by products such as compost. More importantly the use of anaerobic digestion would not be economically attractive without the attainment of a grant. Scenarios 13 – 16 demonstrate this clearly. When a 50% grant is considered in the analysis in conjunction with the higher compost sales value, the IRR rises to 13.8% with payback within 8 years (Table 8.3, Scenario 13). Furthermore, when the waste load is doubled to 4 t week^{-1}, in combination with a 50% grant and a high compost sales value, the use of an anaerobic digestion system becomes financially more attractive (Figure 8.4, scenario 16). At a discount rate of 6% the NPV would be £37,372 with payback within 8 years. Even using a discount rate of 15% in the analysis the project remains profitable with an NPV of £10,704.

The economic viability analysis presented here is based on 100% waste collection efficiency. Presently, less than 1% waste collection efficiency exists, and even with future improvements in waste collection systems, a more realistic value of 30% should be considered. This would not significantly reduce capital costs or operational costs for the patented SWAP system^{TM} but the annual revenue of the system would be reduced. The value of electricity production would be negligible (*c.* £420 per year) and revenue would rely primarily on the sale of compost, which would have an optimum sale value of *c.* £2,000 per year at a rate of £1 per 15 kg bag. If additional costs such as marketing and retail costs of the compost are considered, then the revenue generated would be negligible. Even if a 50% grant were received the system would not be economically viable. At a collection efficiency of 30% with the attainment of a 50% grant and the most optimistic values for revenue generation, the NPV will remain negative at *c.* -£3,000.

Furthermore, it should also be noted that the waste load alone will not guarantee the economic success of the digestion system. Assuming sufficient waste could be supplied, whether through a single large farm or the combination of farms and various other organic waste sources, the fundamental factor governing the success of the system will be the market price received for beneficial by products such as compost. Assuming a gross market price of £4.80 per 15 kg bag (Boyd, 2000), a net price of £1 per 15 kg bag, for the Drummond fish farm example and at a loading of 2 t week^{-1} (as in scenario 7), the NPV would be *c.* £30,000 with pay back within 7 years. Similarly, if a 50% grant were obtained the NPV would be just over £58,000, and pay back would occur in the third year of the project.

Chapter 9

Conclusions

The cage aquaculture industry is dependent on the natural environment for a wide range of "services". These include water, dissolved oxygen and the ability of the immediate environment to remove waste products from the production system. The quality of the immediate environment will thus have a great influence on production and the quality of product. The increasing growth of the global aquaculture industry, in particular the cage production of finfish, will result in increasing pressure on natural resources. This in time may affect the capacity of the aquaculture industry to develop and grow sustainably.

In addition to enhancing the sustainable development of the aquaculture industry, reducing the environmental impact of aquaculture on the environment is of great benefit to the fish farm operator. For example, presently, in Scotland, a predetermined limit of discharge to a freshwater loch governs the annual quantity of biomass the operator can produce (*Section 2.2.1*). Improving the environmental performance of the aquaculture unit could allow fish operators to expand their business. Conversely, greater efforts towards reducing discharges and their effects on the environment could allow fish operators to maintain present production levels as a result of potential stricter discharge regulations being imposed on the industry. Furthermore, public perception is increasingly important for the continued growth and success of an industry. The aquaculture industry must therefore satisfy the consumer that it is producing an "environmentally friendly" product.

The central aim of this study was to enhance the sustainable development of the freshwater cage aquaculture industry by reducing the impact of solid wastes on the environment through undercage collection of waste material and its subsequent biological stabilisation. Subcage collection systems have been reported in literature (*Section 2.3*) but limited data is available concerning their performance in terms of optimal design and suitable environmental conditions, with particular reference to the Scottish freshwater environment.

The subcage collection of waste material from the freshwater cage production of *O. mykiss* was found to be unfeasible at the chosen study site with the present collection system design. Although easy to install, and with no adverse effects on water quality or fish health within the experimental cages, collection efficiency was extremely poor (< 1%). This low efficiency was attributed to the dispersion of wastes from the cage by water currents (*Section 4.6*). Water current velocities at the site were measured and correlated with on site windspeeds measurements at the time of the collection device deployment and were found to exceed the sedimentation rates of both faecal material and feed pellets. Therefore, in order to recover significant amounts of waste using the present undercage collection system design, wind speeds will need to be lower than the mean values recorded at this study site.

Present cage structure design will need to be altered if undercage collectors are to be successfully implemented in lakes with environmental conditions similar to the study site. Waste dispersion was found to occur predominantly in the upper part of the cage, due to natural and possibly fish induced currents. Enclosure of this part of the cage will be required in order to enhance the possibility of achieving vertical deposition of waste material towards an undercage collection device. However, the movement of water through cages is an important part of the replenishment of dissolved oxygen required to

sustain the fish population. At present, satisfactory waste capture from subcage collection systems can only be achieved by complete containment of the cage, resulting in significant economic impacts for the producer due to the need for aeration and pumping equipment to maintain adequate water quality for fish rearing.

The disposal of waste feed and faecal material from the aquaculture production process was successfully achieved through anaerobic digestion. This method of organic waste treatment offers the potential to recycle energy in the form of CH_4 gas, which may in turn be used to generate heat and/or electricity. An initial investigation revealed that a significant proportion of the waste could be stabilised in terms of its oxygen demand. Temperature did not appear to affect the degree of biological stabilisation significantly but clearly influenced the production of CH_4 (*Section 5.1.3*). For optimum performance in terms of CH_4 yield the mesophilic and thermophilic temperature ranges should be used. Experimental data also revealed that the anaerobic process was capable of inactivating a common fish bacterial disease at both thermophilic and psychrophilic temperatures. Furthermore, analysis of solids data from the digestion process showed that the solubilisation of waste material was the rate limiting step in the biodegradation process.

The addition of nutrients to supplement the digester feed proved ineffective in enhancing the process (*Section 6.1.3*). Ultrasonication as a pre-treatment method for the psychrophilic anaerobic biodegradation of aquaculture waste solid effluents did show a significant enhancement of the digestion process. There was a 10% increase in both oxygen demand and reactive P reduction. More significantly, daily biogas production increased by approximately 55%, with a corresponding increase in CH_4 content. The increase in digestion performance showed that pre-treated waste when fed to the reactor increased the transfer of organic compounds from the waste solids to the aqueous phase.

These results confirm the hypothesis that hydrolysis is the rate limiting step in the anaerobic digestion of aquaculture wastes.

Analogous to the pre-treatment of waste material in order to enhance the digestion process, post-treatment through biofiltration was feasible at organic loading rates of 0.53 and 1.04 kg COD m^{-3} day^{-1} (*Section 6.4.3*). The nitrification of NH_4-N to NO_3-N was not inhibited by the increase in loading rate, although at the higher loading rate there was *c.* 8 and 10% increase in effluent SS and BOD respectively. The combination of anaerobic digestion and a simple aerobic biofiltration unit was shown to significantly improve digester effluent quality.

The economic viability of anaerobic digestion for the treatment of aquaculture waste effluents was evaluated and was found to depend primarily on the quantity of waste available for digestion (*Section 8.4*). Financial viability will also be strongly influenced by the ability or motivation to maximise the utilisation of the beneficial products from the digestion process, particularly the production of saleable compost. In turn, the market price received for the sale of compost is fundamental to the success of the digestion system.

In the case of the study site used for monitoring the collection system on Loch Earn, the economic viability of the anaerobic digestion system was largely dependent on the efficiency of the waste collection system. Assuming 100% waste collection and the attainment of a 50% grant in conjunction with the maximum use of digestion by products, primarily the sale of compost, the project would be economically viable, although financially, an unattractive investment for the farm.

At present the waste collection system operates at <1% efficiency. With future improvements, a more realistic figure of 30% collection efficacy could be assumed. The use of an anaerobic digestion system as a means of waste management for aquaculture waste effluents for a single cage farm (300 t yr^{-1} production) would therefore not be financially attractive, primarily due to the small quantity of waste material available for digestion. The process will only become economically attractive if there are a number of farm units or other sources of organic waste working together.

As a possible alternate route therefore, the co-digestion of aquaculture waste effluents with cattle slurry was investigated. Although temperature fluctuations hindered the analysis of data, it was still evident that co-digestion of cattle slurry (*Section 6.3.3*) had no adverse impacts on the digestion process. Cattle slurry would therefore prove a valuable source of additional organic material in order to enhance the economic viability of anaerobic digestion for the treatment of aquaculture effluents at Scottish fish farms.

In summary, therefore, it can be concluded that due to excessive water velocity speeds, the undercage collection of aquaculture wastes in a freshwater lake environment with environmental conditions similar to that of the study site is not possible. However, anaerobic digestion as a means of waste treatment and disposal for aquaculture effluents is feasible. Pre-treatment of the dewatered waste is not necessary for digestion, but due to the particulate nature of the waste will enhance the process, particularly the production of CH_4, which may be used as a renewable energy source. If anaerobic digestion as a waste treatment method for aquaculture waste on typically sized fish farms is to be economically viable, the utilisation of its by products and external funding will be required. An additional organic waste stream such as cattle slurry would make such a system more financially attractive.

Chapter 10

Future Research

Future directions for research that follow from this thesis include further evaluation of undercage collection systems with emphasis on alternative aquaculture cage design. The performance of the collection system in this study was hindered primarily by the velocity of lateral water currents. However, a change in cage design, such as the incorporation of some form of intermittent barrier to restrict flow, would aid in containing the wastes within the cage during feeding and periods of peak gut evacuation and may allow for a reduction in waste dispersion and hence improve collection efficiency. Such work would need to consider the behavior of the fish species during feeding. Although some work has been carried out in this area, such as *S. Salar*, social behavior within fish has been demonstrated to be species dependent and similar work is required for *O. mykiss*. The fundamental advantages of cage culture over land based systems should be considered in such a study. Care most be taken not to inhibit the water quality of the cage system, such as DO replenishment, while ensuring capital costs remain low.

In addition, the performance of the undercage collection system assessed in this study was site specific. There is a need to investigate the use of such a system in other sites which may not be as exposed as Loch Earn. For example, a more sheltered site would result in lower wind velocities and hence lower water current velocities. This may allow for greater vertical deposition of wastes and enhance the collection efficiency.

Similarly, there is a greater need for the direct measurement of water velocities in the lake environment. Much work has been carried out in this area with respect to marine

aquaculture activities thus helping to assess more accurately the impact of aquaculture on that environment. Further understanding of water hydrodynamics in the lake environment would similarly aid to evaluate the impact of freshwater aquaculture and allow for more suitable site selection in the first instance.

With regard to the treatment of aquaculture wastes, this study demonstrated the feasibility of anaerobic digestion. However, the potential of this technology to inactivate both fish viral and bacterial diseases needs to be further clarified. Due to limited resources, it is unclear from this study if anaerobic digestion was successful in the complete destruction of a *Y. ruckeri*, a common fish bacterial disease. Therefore, future research is required to elucidate this.

Furthermore, as a precursor to the treatment of aquaculture wastes, research regarding the dewatering of such wastes is required. Due to the poor performance of the collection system, methods for the reducing the water content of aquaculture wastes were not evaluated. Previous studies have documented the use of screen filtration (for land based systems) but this technology is known to be troublesome and high maintenance is required due to mechanical nature of the devices. This would not be particularly ideal for cage aquaculture where dewatering at the point of collection (i.e. after pumping to surface) would be more practical and less laborious.

Finally, this thesis revealed that the use of anaerobic digestion as a means of waste treatment for a typically sized Scottish aquaculture farm is not financially viable. Additional organic waste would be required to increase the organic loading of the digester and hence increase the production of beneficial by-products such as biogas and compost. Although cattle slurry was demonstrated in this research to be feasible for co-digestion with aquaculture waste, other sources of organic waste should be assessed. For example,

the use of seaweed in coastal locations or fish offal where fish processing may be an integral part of aquaculture activities.

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